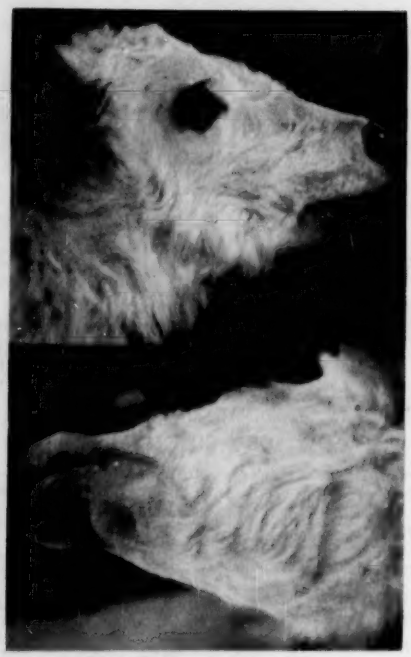
The Journal of HEREDITY



UNDERDEVELOPED MANDIBLE
(SEE PAGE 165)

Dwarf Lespedeza
Polydactylous Mice
Supernumerary Chromosomes
Somatic Color Mutations in Alfalfa
Cytological and Genetical Effects of Endothal

Volume 47 JULY-AUGUST, 1956 Number 4

The American Genetic Association

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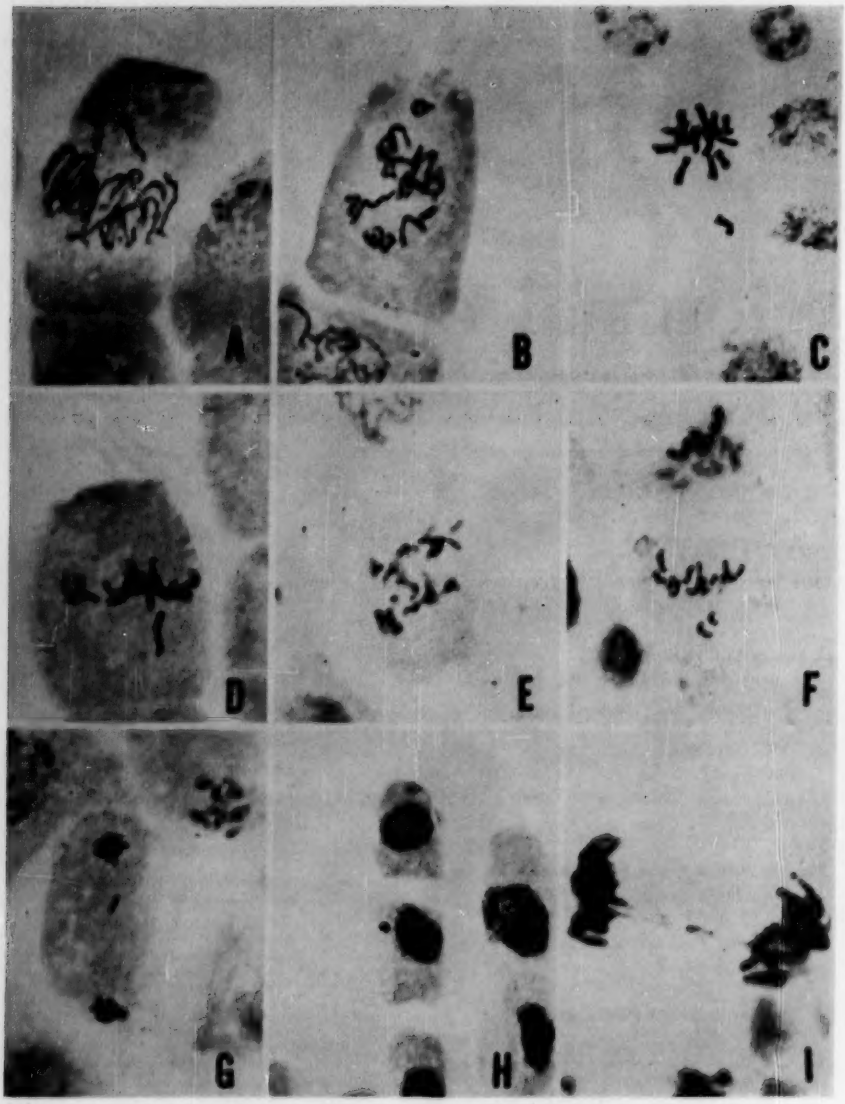


LAGGING CHROMOSOMES

Figure 1

A and *B*—Laggards at prophase; *C*—laggard at prometaphase; *D*—laggard at metaphase; *E*—asynchronous anaphase; *F*—laggard at anaphase outside of spindle area. Note chromatid separation. *G*—Laggard at telophase; *H*—micronuclei resulting from "lost" laggard; *I*—bridge resulting from dicentric chromosome.

CYTOLOGICAL AND GENETICAL EFFECTS OF THE DEFOLIANT ENDOTHAL

S. M. Wilson, Arlyn Daniel and G. B. Wilson*

DURING the past seven years a number of antibiotics, organic herbicides and insecticides have been tested in this laboratory for antimitotic activity and radiomimetic action. The three major categories of effect may be described as follows:

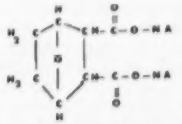
1. *"C-mitotic" substances.* These act in essentially the same way as colchicine. In sufficient concentration, anaphase movement is halted and cytokinesis normally inhibited or prevented. Also movement out of prometaphase does not occur. Prophase movement is generally normal except at low doses or on recovery.[1, 4, 7]

2. *"Prophase poisons."* Substances in this category (of which there are many) interfere with prophase movement in such a way that the chromosomes spread out around the nuclear wall, contract and become telomorphic without moving into typical prometaphase or metaphase configurations. At sublethal doses, however, anaphase movement is normal where a nucleus was at metaphase at the time of exposure. Also all such compounds which we have tested have had a marked inhibitory action on entrance of nuclei into division[6].

3. *Radiomimetic substances.* Such compounds can induce *bona fide* chromosome fragmentation followed by structural rearrangement in a fashion at least reminiscent of radiation. A number of true mutagenic chemicals, though apparently not all, fall into this category[3].

Of the more than thirty chemicals screened in this laboratory most appear, even from primary screening, ascribable definitely to the second category. One, λ-cyclochlorohexane is certainly in the first category[4]. None is ascribable to the third category with certainty. However one compound, Endothal, gave results which led us to believe that it might be more nearly related to substances of the third category than to any other. The present paper is concerned both with the cytological and genetical effects of this compound in its commercial form.

Endothal is a defoliant, the active ingredient of which is 3,6 disodium endoxohydrophthalate at a concentration of 19.2 percent. The structural formula is,



Cytological Studies

Roots of *Allium cepa, Vicia faba* and *Pisum sativum* var. *Alaska* were treated for varying times with various concentrations. While results from all three plants are comparable, those obtained by using Bowen and Wilson's[1] "Pisum test" are most complete and will be referred to here exclusively. Roots between two and three centimeters long were treated with concentrations of 1, .5, .2 and .1 ppm active ingredient for six and 24 hours with frequent samples taken for cytological analysis. Controls were run simultaneously in all cases.

The mitotic index. As has been the case with all prophase poisons studied, the mitotic index, after an initial rise, fell off markedly and at 1 ppm virtually reached zero at 24 hours. (See Figure 2*A-F*) The initial rise in this, as in other cases, appears to be the combined result of prophase stalling and the progression of very early prophases to scorable prophases. However, Endothal, unlike typical prophase poisons such as actidione, does not appear to prevent prophases from reaching more or less orderly metaphase and anaphase configurations. Metaphases did show a tendency to clump especially at the high dosages (1 and .5 ppm) but seldom to a degree which prevented more or less normal separation of chromatids.

*Contribution number 56—1 of the Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan. This work was carried out under the Office of Naval Research contract number 164203 and with the aid of funds supplied by the All College Research Committee. We wish to express our gratitude to Dr. Allen S. Fox and the Department of Zoology for making *Drosophila* stocks and facilities available and to Mr. P. G. Coleman for assistance with illustrations.

The production of "laggards." In Endothal treated material, many cells at all stages of mitosis contained one or more chromosomes, the movements of which deviated from the main mass and were often lost. (Figure 1*H*). This effect serves in itself to distinguish Endothal from any other substance tested. Such deviant chromosomes have been termed "laggards," although the term is not always appropriate since they sometimes appear to move either prematurely or in the wrong direction. In prophase, for instance, some "laggards" appear to be actually expelled from the nucleus, while in other cases they appear to be simply left behind (Figure 1 *A*, *B* and *C*). In metaphase, anaphase and telophase (Figure 1 *D*, *F* and *G*) they may be either in the spindle area or well outside of it. In any event kinetochore cleavage appears to be normal and synchronized regardless of the position of the omitted chromosome relative to the spindle (Figure 1*F*). "Lagging" chromosomes are found occasionally, of course, in tissue treated with other compounds as well as in controls. However, the frequency of affected cells (up to 40 percent) is much higher than in any other case with which we are acquainted.

Quantitative analysis of "laggard" data poses a number of difficulties arising from various partial correlations with other changes such as mitotic index and stalling effects. Furthermore, the decision as to which and how many chromosomes should be designated as "laggard" is not equally easy at all stages. In this regard notable difficulties are encountered at early prophase where insufficient movement has occurred to allow differentiation as to "lagging" and "non-lagging"; at metaphase in those cases where clumping obscures organization and in asynchronous anaphases (Figure 1*E*) where movement, though bipolar, appears to be erratic. Nevertheless, despite these difficulties, several definite trends are clearly demonstratable.

Prophase: In all cases (Figure 2*A-F*) the percentage of prophase cells containing "laggards" reaches a maximum at four hours' treatment and then decreases rapidly. However, this trend must be viewed in the light of two related facts: (1) At the time of maximum effect the average prophase is about mid-prophase, since few or no new prophases are being initiated and (2) by six hours there are virtually no prophases. In the "recovery" experiments (Figure 2*B* and *D*) when new prophases begin to appear a perceptible percentage again show "laggards." The apparent prophase trend is open to a number of equally likely interpretations, none of which can be substantiated by the present data. From the "recovery" experiments one definite conclusion appears to be strongly indicated; namely, that preprophase or antephase is a sensitive stage since the effect is shown in prophases emerging long after the stimulus has been removed.

Prometaphase and Metaphase: The trends for these stages (Figure 2*A-F*) are likewise open to various interpretations. The fact that the percentage of cells showing "laggards" at these stages is higher than in prophase may mean either that "laggards" originate at these stages or may only reflect the relative ease of decision concerning their presence. The fact that the "laggard" peak is more or less coincident with the "stalling" peak favors the latter interpretation since the bulk of the prometaphase and metaphases have arisen from those cells which passed through prophase in treatment. By the end of 24 hours in continuous treatment the percentage of cells with "laggards" does not differ significantly from the initial peak except in the 1.0 ppm run in which the number of dividing cells at 24 hours is so low that the points cannot be considered very reliable. In both "recovery" runs (Figure 2*B* and *D*) there is still an appreciable percentage of affected cells.

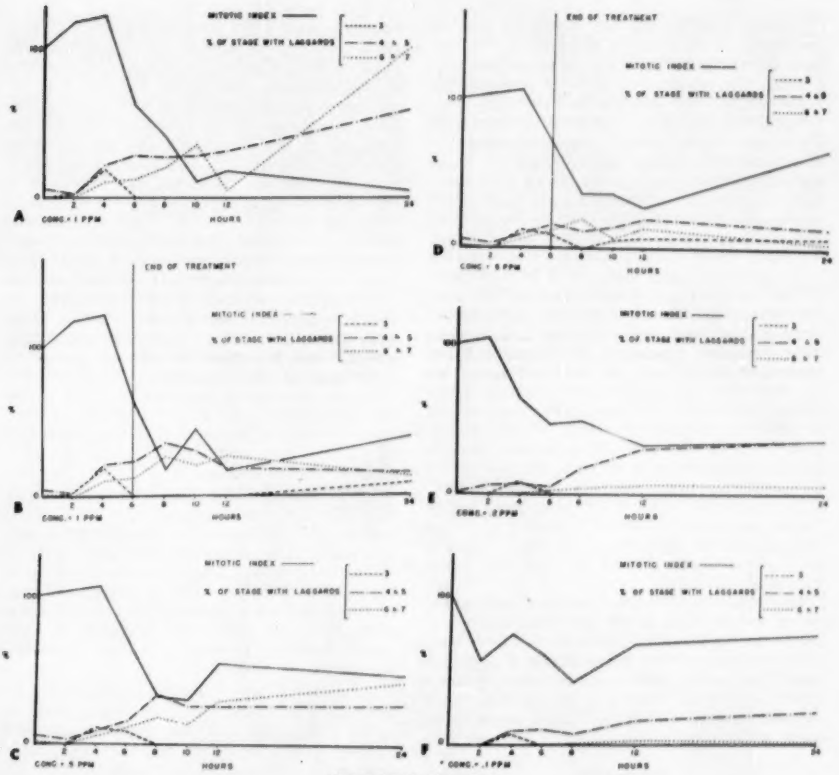
Postmetaphase: The trend so far as postmetaphase "laggards" are concerned is similar to metaphase with the peak coming somewhat later. It is again impossible to determine whether "laggards" are initiated at these stages since some chromosomes which were definitely lagging or misaligned earlier may "catch up" while others which were imperceptibly displaced may come to be farther behind.

Despite this confusion several conclusions appear obvious. After treatment with Endothal an appreciable number of chromosomes in dividing cells do not conform in movement pattern to the majority and, in general, 24 hours treatment is no more potent in this regard than six. Also, we feel that the data are best interpreted on the basis that preprophase, and perhaps, very early prophase are the stages of primary effect.

Some data have been compiled on the average number of "laggards" per affected cell for the various stages. Since lagging is a matter of degree such data are not very satisfactory. However, if we consider only otherwise well organized prometaphase and metaphase nuclei the number of "laggards" seldom exceeds four and is more commonly one or two. In contin-

TABLE I. Average number of "laggards" per affected cell at prometaphase and metaphase

Concentration Hours Treated	1.0 ppm	0.5 ppm	0.2 ppm	0.1 ppm
0 hrs.	0.0	0.0	—	—
2 "	1.5	1.3	1.8	—
4 "	1.7	1.5	1.2	1.2
6 "	1.7	1.5	1.6	2.0
8 "	2.1	1.8	1.3	2.1
10 "	2.0	2.5	—	—
12 "	1.4	2.0	2.1	1.7
24 "	1.8	2.1	2.1	2.6
Hours Recovery from 6 hours Treatment				
2 hrs.	2.5	2.1		
4 "	2.1	1.3		
6 "	1.5	1.4		
18 "	1.4	1.1		



MITOTIC INDEX

Figure 2

A-F—Graphical representations of various concentrations of Endothal. Numbers refer to stages of mitosis as follows: 3, prophase; 4, prometaphase; 5, metaphase; 6, anaphase and 7, telophase.

uous treatment there is also a slight tendency for the average number to increase up to eight or 10 hours and then remain more or less constant. In "recovery" experiments the average number decreases slightly by 24 hours (Table I). While there is no evidence that any particular chromosome is more prone to be affected than the rest, this possibility has not been thoroughly checked.

To us this failure of some chromosomes to move in concert with the majority implied direct or indirect damage to the chromosome by this treatment which in turn suggested the possibility of genic damage. It was therefore considered well worth testing this compound for mutagenic activity.

Genetic Studies

Since preliminary tests using the "attached X" *Drosophila* system gave suggestive if not clear cut results, it was considered worth while to investigate the relationship between the frequency of X chromosome lethals and Endothal treatment by using the much more reliable "Muller-5" technique. To this end the following experiments were performed:

1. *Vapor treatment.* Males of the Oregon-R strain were placed in a treatment flask. Air which passed through a container of commercial Endothal was drawn or forced into the treatment chamber over a period of 24 hours. Control males were kept under essentially the same conditions as the treated ones for the same time. Appropriate serial matings were made to Muller-5 females over a nine-day period. Three brother-sister paired matings were made from each F_1 bottle and the F_2's produced scored for presence of sex-linked lethals as indicated by absence of red eyed males. Only bottles showing complete absence, and with sufficient flies to obviate the possibility that the

deficiency was merely the result of random fluctuation, were recorded as positive. Furthermore all suspected lethals were tested and confirmed. The results for controls are given in Table II and for treated in Table III. According to these data treatment of males with Endothal vapor raises the frequency of X-chromosome lethals about ninefold. In a number of cases the F_2 yield of red eyed males was well below expectation, which probably implies the presence of sublethals. No attempt was made to count these so that the mutation frequency given must be considered as minimal.

2. Larval feeting. Oregon-R females were allowed to lay eggs on a media in which various concentrations of Endothal were incorporated. Hatching larvae were fed this medium until pupation. Surviving males were tested against Muller-5 stock as described above and the frequency of X-chromosome lethals determined. At Endothal concentrations in excess of 250 ppm no eggs hatched. Hatching was poor at both 100 and 250 ppm and notably below normal down to 10 ppm. Because of the comparatively small numbers involved the results from both 100 and 250 ppm were added. Again, as shown in Table IV, feeding of larvae was associated with about the same increase in X-chromosome lethals as was the vapor treatment of adults.

One apparent difference between feeding and vapor treatment is in the increased number of sterile F_2 bottles after the former. There were about three times as many sterile F_2 cultures from the feeding series as from controls (See Table V), while there was no significant difference between F_2's derived from vapor treatment and controls.

Discussion

From the cytological reactions it is quite obvious that Endothal is not a "C-mitotic" substance and aside from its tendency to suppress mitosis, its effects do not agree with those of typical prophase poisons. It is related to radiomimetic substances only in that the deviant behavior of some of the chromosomes may be interpreted as reflecting chromosome damage. A few fragments and an occasional dicentric (Figure 1*f*) indicative of translocation were found. However, the frequency of such events was too low to consider significant. Even on the basis of the most conservative scoring of results, Endothal must be classed as a mutagen when administered either as a vapor or a food ingredient. While we are of the opinion that Endothal is less effective as a mutagen than such chemicals as the nitrogen mustards, lack of suitable standards makes adequate comparison difficult. However, if we assume that the methods of administration are reasonably comparable, then Endothal does appear to be less effective in practice. It may be pertinent that the cytological effects are also comparatively mild in terms of chromosome damage.

Such examination as has been made of salivary gland chromosomes to date (J. C. Hadder, unpub.) indicates that the induced lethals are not major deletions, rearrangements or chromosome loss. The increased sterility of F_2 cultures from the feeding experiment may be correlated with chromosome loss but seems more likely to reflect the presence of autosomal lethals. However, neither possibility has yet been tested.

Two points seem worth stressing. In the first place it would appear probable that any agent which damages the chromosomes even slightly might well turn out to have positive mutagenic potential. Unfortunately very few of the milder mutagens have been adequately tested. In the second place, Endothal is a commercial preparation which has been used

TABLE II. The occurrence of sex-linked lethals in controls

Exp. No.	No. of X-chromosomes Tested	No. of Sex-linked Lethals	% of Sex-linked Lethals
I	64	0	0
II	648	0	0
VIII	411	1	0.232
IX	368	0	0
Total	1,511	1	0.066

TABLE III. The occurrence of sex-linked lethals from aerosol or vapor treatment of adult males with Endothal

Exp. No.	No. of X-Chromosomes Tested	No. of Sex-linked Lethals	% of Sex-linked Lethals
I	128	1	0.828
II	1,122	5	0.378
VIII	878	7	0.797
Total	2,128	13	0.558

TABLE VI. The occurrence of sex-linked lethals from the feeding of larvae on Endothal media

Exp. No.	Conc.	No. of X-Chromosomes Tested	No. of Sex-linked Lethals	% of Sex-linked Lethals
IX	250 ppm	421	2	0.475
	100 ppm	361	2	0.554
Total		782	4	0.512

TABLE V. Percentage of sterile F_2 cultures from larval feeding

Exp. No.	Conc.	Total Cultures	No. Sterile	% Sterile
VIII	cont.	446	15	3.4
IX	cont.	383	15	3.9
Total		829	30	3.6
IX	250 ppm	460	39	8.5
	100 ppm	409	48	11.7
Total		869	87	10.0

rather extensively as a defoliant. If any significant proportion of such widely used preparations should have mutagenic activity and if their effects are cumulative then the genetic results for man and other organisms may well be unpleasant or even catastrophic.[5] Certainly the indiscriminate use of mutagenic chemicals is as much to be deplored as careless use of radiation.

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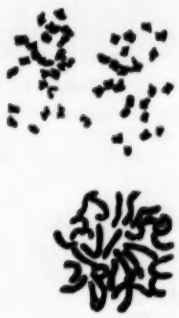

A DWARF MUTATION IN SERICEA LESPEDEZA

C. H. Hanson and W. A. Cope*

A dwarf mutant (Figure 3) was noted in survey plantings of sericea lespedeza (*Lespedeza cuneata* Dumont, G. Don). The dwarf was characterized by shortened internodes, small leaves, and moderately light green color of foliage. The mutant bred true for these traits. Insofar as the authors are aware, this is the first dwarf mutant reported for this diploid species.

Crosses between the dwarf line and 10 normal plants yielded normal-appearing F_1 hybrids. Selfed seed (seed from cleistogamous flowers) was harvested from each of the 10 F_1 hybrids and planted in the greenhouse in

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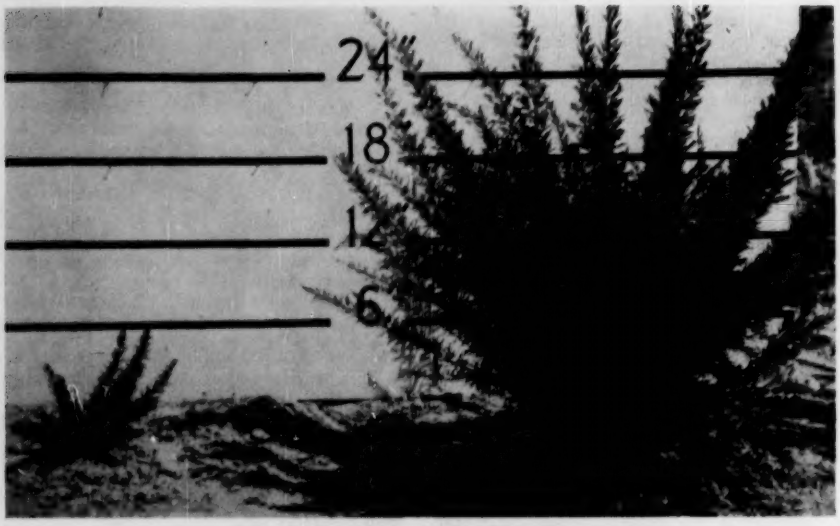

DWARF AND NORMAL LESPEDEZA

Figure 3

A dwarf plant (left) and a "normal" plant of Sericea lespedeza. The dwarf mutant was characterized by shortened internodes, small leaves, and moderately light green color of foliage. (One year old plants photographed June 2, 1953.)

1 × 1 inch plant bands on March 26, 1954. The seedlings were about 1½ inches in height when they were transplanted to the field a month later. Differences between dwarf and normal plants became apparent soon after transplanting, and on July 1 differences were sufficiently distinct for scoring. The percentage of dwarfs on that date ranged from 16 to 26 percent for the 10 F_2 progenies (Table I). Chi-square tests showed a close fit for a 3:1 segregation in one-half of the progenies, assuming that dwarfness was conditioned by a single recessive gene. The other five progenies, and the population obtained from pooling progenies, however, deviated significantly from a 3:1 ratio; there were an excess of normal and a deficiency of dwarf plants.

Twelve percent of plants died between the time of transplanting and July 1. Unfortunately, it was not possible to determine accurately the proportion of plants lost in each of the two classes so as to include them in the scoring on July 1. A second scoring on August 26, which followed a drought period, revealed that a further reduction in stand of four percent had occurred since July 1, with plant losses occurring only in the dwarf class. In view of the latter, it is postulated that a proporionately greater number of dwarf than normal plants died between the time of transplanting and July 1, and that dwarfness was conditioned by a recessive gene in the homozygous condition.

TABLE I. Dwarf and normal plants in segregating F_2 progenies tested for goodness of fit for a 3:1 ratio

Cross	Normal	Dwarf	Total	Dwarf %	χ^2
1	151	33	184	18	4.51*
2	112	28	140	20	1.61
3	161	30	191	16	8.35†
4	134	42	176	24	.07
5	131	29	160	18	3.68
6	127	27	154	18	4.19*
7	132	23	155	15	8.05†
8	113	39	152	26	.01
9	127	25	152	16	5.48*
10	138	34	172	20	2.24
Crosses pooled	1326	310	1636	19	31.63†

*χ^2 significant at 0.05
†χ^2 significant at 0.01

THE STATUS OF SUPERNUMERARY CHROMOSOMES IN DIABROTICA

After A Lapse Of Fifty Years

STANLEY G. SMITH*

WILD populations carrying supernumerary chromosomes are relatively well known in the Insecta[3, 4, 10] presumably because of the considerable attention this subphylum has attracted cytologically. Supernumerary chromosomes are so named because some individuals of a species lack them entirely. They are recognizable when they stain differentially; when they occur in odd numbers; and when, as they usually do, they behave irregularly during meiosis. Since they appear to have little or no morphological effect on their carriers they are said to be genetically inert and to be composed of heterochromatin, as opposed to the euchromatin of the genetically "active" genes. This absence of any marked outward effect, coupled with their presumed persistence over a number of generations, renders attractive the hypothesis that they play an important but obscure role in internal cellular physiology—a role that, if they are not selectively eliminated or otherwise numerically reduced in the course of time, must be regarded as being indispensable for the species, though not for the individual.[1]

Some 50 years ago, during a general survey of beetle cytology, Stevens[8] encountered two chrysomelids, *"Diabrotica soror* and *D. 12-punctata,"* in which different individuals were found to possess from zero to four extra chromosomes. Her two "species," which were collected from Mountain View, California, and Bryn Mawr, Pennsylvania, respectively, are now recognized as being two subspecies, *D. undecimpunctata undecimpunctata* Mann. and *D. u. howardi* Barber. Her examination of 100 males of each disclosed the presence of supernumeraries in about 50 percent of them, their distribution being statistically the same in each sample.

Since the species has been subjected to no further investigation and as it provides a made-to-order opportunity for studying the population dynamics of supernumerary chromosomes, steps were taken to solicit large collections from both California and points in the east. Two such collections have been received, both from the east.

Material and Methods

One of the samples of *D. u. howardi* was obtained from the Chatham area in southern Ontario through tne kindness of Mr. John A. Begg, Entomology Laboratory, Chatham; the other from near East Lansing, courtesy of Dr. J. R. Hoffman of Michigan State University. These provided preparations from 127 and 115 males, respectively, but the females in the samples failed to show divisions.

The paired testes, fused together and lying asymmetrically to the right of the intestine, are orange or yellowish and thus readily dissected from the ripped dorsal side after pinning forward the elytra and wings. Following fixation in Smith's modification of Kahle's fluid for a couple of minutes, the testes were transferred to a drop of 45 percent acetic acid on an albuminized slide and torn into a dozen pieces, which were then arranged systematically under a glass cover slip. After warming the slide over an alcohol flame the pieces were flattened by applying gentle pressure through a layer of blotting paper and heated three or four times more. The cover slip was carefully pried off in 50 percent alcohol and staining carried out in Feulgen (14 min. hydrolysis; 2 hrs. leuco-basic fuchsin) and light green. Photomicrographs were taken on contrast process panchromatic film at an original magnification of 1,500 ×;

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A
B
C
D
E
F

they are reproduced here at 1,900 ×. I am indebted to Mr. D. C. Anderson for assistance with these photomicrographs.

Observations

The first 100 Chatham males to give satisfactory counts had from 19 to 24 chromosomes, that is, from zero to five supernumeraries as follows: 21 with none (Figure 4*A* and *E*); 36 with one (Figures 4*B* and *F*, 5*A*, *B* and *C*); 24 with two (Figures 4*C* and 5*D*); 11 with three (Figure 5*E* and *F*); 7 with four (Figures 4*D* and 6*A* and *B*); and 1 with five (Figure 6*C*). The East Lansing males comprised only five numerical types with from zero to four extra chromosomes, there being 22 individuals with none; 42 with one; 23 with two; 11 with three; and 2 with four. Thus the Chatham males had a supernumerary-chromosome frequency of 1.50, the East Lansing males one of 1.29, or, together, 1.395 supernumeraries per male.

The chromosome number of males could be readily determined in first metaphases, which occurred commonly on almost all of the slides, but only with difficulty in spermatogonial mitoses (Figure 4*A-D*), which were more rarely encountered and in which the small extra chromosomes were often presumably concealed by the larger ordinary chromosomes. When visible, in especially well spread metaphases, however, they usually tended to lie nearer the center of the plate (Figure 4*C* and *D*), as is depicted also by Stevens (*v.* her Figures 28, 49, and 67). At first metaphase the supernumeraries were clearly seen to be of two types (e.g. Figures 4*F*, 5*B* and 6*C*), both small but one considerably smaller than the other. Spermatogonial metaphases disclosed the larger of the two to be metacentric, V-shaped elements about the length of one arm of the ordinary chromosomes and somewhat narrow (Figure 4*B-D*), and the smaller to be about half the size of the larger ones and apparently rod-shaped.

So far as could be ascertained and disregarding certain erroneous interpretations she made, for example concerning a continuous spireme, there appear to be only minor differences in chromosome behavior from the description given by Stevens. In both, the supernumeraries are condensed (positively heteropycnotic) throughout the prophase of meiosis and are never joined to the ordinary autosomes. Where more than one are present, association between them in pairs or higher multiples (Figures 5*F* and 6*B*), although seemingly more frequent in the present material (5.6 percent), than in Stevens', appears to be the result of the nonspecific attraction characteristic of heterochromatin and a relic of their former juxtaposition during spermatogonial mitoses and early meiotic prophase. Occasionally, however, metaphase configurations were observed that suggest that the associations may have been through chiasmata (Figure 5*F*), and in certain of the metaphases and anaphases the supernumeraries were arranged *vis-à-vis*, as though having undergone disjunction one from the other (Figures 5*D* and *E* and 6*A*). In agreement with Stevens' description, association was not infrequently (5.2 percent herein) observed between a supernumerary and the X chromosome (Figure 5*C*). This is not unexpected, since the X is likewise heterochromatic in the male and is closely grouped with the supernumeraries in the pachytene bouquet. But the association was not normally accompanied by congression on the equator nor by co-orientation and, therefore, does not necessarily condition co-ordinated disjunction, as it does in autosomal bivalents. Otherwise the current observations all point to the supernumeraries being autonomous in behavior, either dividing at the first anaphase (Figure 6*D*) or passing at random to the poles (Figure 6*E*) and dividing at the second (Figure 6*F*).

The major difference between my material and that in Stevens' account concerned the relative frequencies of supernumeraries of different size. She found only one individual, and that from the east, with a single, much smaller supernumerary among the 200 males examined. Grouping the two samples studied here, 11.0 percent of the beetles carried small supernumeraries, the latter constituting 12.1 percent of the total number of extra chromosomes observed.

Discussion

The frequencies of males with different numbers of excess chromosomes in the Chatham and East Lansing collections are given in Table I along with those determined by Stevens. The results of the chi-square tests, given there, establish with virtual certainty that the two samples in the present study are not significantly different from each other in chromosome constitution and that both differ markedly in this respect from the 100 Bryn Mawr males that were examined nearly 50 years ago. It will be seen from the table that the mean number of supernumeraries per male was 0.67 at Bryn Mawr in 1907 as against 1.40 at Chatham-East Lansing in 1954. The distributions found by Stevens and by myself

CHROMOSOMES OF *DIABROTICA U. HOWARDI*

Figure 4

← *A-D*—Spermatogonial metaphases with zero, one (at 9 o'clock), two (small V's at center), and four (3 V's and 1 rod around center) supernumerary chromosomes, respectively. *E* and *F*—First metaphases with 9_{II} + X (at lower pole) and 9_{II} + X (below plate) + 1S (S = large supernumerary, at right), respectively. Note: In some of these photographs, and others in Figures 5 and 6, there are small spots caused by extraneous matter; they can be readily distinguished by the surrounding light-ring.

A
B
C
D
E
F

being accepted as representative, the question arises as to whether a cytological mechanism is operating so that the frequency of supernumeraries is being gradually increased, having more than doubled over the past 50 years. This seems unlikely, since they have doubtless been handed down from the common ancestor of the two subspecies, yet had failed to attain different frequency distributions by 1907. Certainly no cytological mechanism was detected *in the male* that would account for the net increase observed; equally none was revealed that would tend to lower their overall frequency.

That the greater number of supernumerary chromosomes present in the current materials is not ascribable to the higher number of the smaller ones is clear from their frequency being far too low (ca. 12 percent) to account for the better than 100 percent excess over Stevens' Bryn Mawr total. The V-shape of the larger supernumeraries, the rod shape of the smaller ones, and their relative sizes immediately suggest that the latter may have originated through misdivision of the centromere of the former, as perhaps occurs in the turbellarian *Polycelis tenuis* Ijima[4]. Whether the metacentrics are true isochromosomes with homologous arms, as White[9] interprets some to be in trimerotropine grasshoppers and Müntzing and Lima-de-Faria[5] have demonstrated by chromomere analysis to be the case in certain of the "B chromosomes" of *Secale cereale*, cannot be ascertained in *Diabrotica* because of their strong heteropycnosis at pachytene.

The X chromosome, by dividing only once, is transmitted to exactly one-half of the sperm, in accordance with the requirements of a 50:50 primary sex ratio. The supernumeraries likewise usually segregate at random, although they may divide at either anaphase. If, of course, the 5.2 percent that were seen in association with the X chromosome actually disjoin from it, a corresponding reduction in number of supernumeraries will arise in the X-bearing sperm and be balanced by a comparable increase in those in the non-X-bearing sperm. As a consequence, the females of a population may carry somewhat fewer supernumeraries than the males, but with free mating this would in no way cause changes in number from generation to generation. It was probably this contingency that Stevens had in mind when she concluded (p. 458) that the four classes of sperm produced by males with one extra chromosome may be quite unequal. But if so, it conflicts with her statement (p. 462) that ". . . the small heterochromosomes seem to be as likely to go to the spermatozoa which receive the odd chromosome (x) as to those which lack it. . . ."

Whatever the influence on disjunction of associations between the supernumeraries and of them with the X, these observations make it quite clear that the maximum number of extra chromosomes carried by the sperm is equal to the number in the individual. Thus the range in the sperm is from zero to five for the Chatham sample and zero to four for that from East Lansing, in each case with or without the X chromosome. The factors that operate in the male to ensure constancy in total number of supernumeraries from generation to generation are: (1) their capacity for division at either the first or the second anaphase and (2) their inability to divide at both. Those that allow increase within individual progeny are: (1) the failure of supernumeraries to pair at meiosis; (2) their consequent random distribution during the two anaphases; and (3) subsequent free recombination.

Neither Stevens nor I have, for technical reasons, been successful in establishing counts for females, but they must obviously carry supernumeraries, for in segregation the latter are largely, if not entirely, independent of the X chromosome. Now, where examined, the two meiotic divisions in the egg are linearly arranged, so that lagging chromosomes, such as those that fail to pair, are liable to exclu-

TABLE I. Distribution of supernumerary chromosomes (S) in *D. undecimpunctata* males from four localities

Source of sample	Number of males with						χ^2	D.F.	P	S/♂
	0S	1S	2S	3S	4S	5S				
Mnt. View	48	33	15	3	1	0	0.907	2	.70–	0.76
Bryn Mawr	51	35	11	2	1	0	21.836	2	.01–	0.67
E. Lansing	22	42	23	11	2	0	1.631	3	.70–	1.29
Chatham	21	36	24	11	7	1				1.50

FIRST METAPHASES IN *DIABROTICA U. HOWARDI*

Figure 5

← *A*—9_{II} + X (below) + 1S (above). *B*—9_{II} + X + 1s (s = small supernumerary, by leftmost bivalent). *C*—9_{II} + XS (associated below, S paler). *D*—9_{II} + X (below) + 2S. *F*—9_{II} + X (below) + 1S + 1S"II".

A
B
C
D

sion from the female pronucleus. If this holds in *Diabrotica* and the supernumeraries fail to pair, they must suffer considerable elimination in each generation. If, on the other hand, they are not heteropycnotic in the female and pair in twos, as the X chromosomes do in other species of beetles[7] and the supernumeraries do in *Polycelis tenuis*[4], loss is to be expected only, or mainly, when they are present in odd numbers. In other words, females with two or four supernumeraries might produce eggs with constant numbers of extra chromosomes and those with one or three might produce eggs largely with zero or one. If so, such a mechanism would tend to favor numerical stability among progeny of even-numbered females and to reduce variability among those from odd-numbered females.

Knowing that the supernumeraries move essentially at random during spermatogenesis, if one assumes that they associate in pairs during oogenesis, it is relatively simple, although tedious, to calculate the expected constitution of the progeny from any hypothetical population. Thus a freely interbreeding group consisting of males and females of which 50 percent have zero, 33 percent have one, 13 percent have two, 3 percent have three, and 1 percent has four supernumeraries should have a grouped progeny consisting of 58.26, 31.46, 8.54, 1.52, 0.20, 0.02, and 0.0006 percent individuals having from zero to six extra chromosomes. In the next generation, following random mating, the percentage in the zero class should increase to 70.41. If, however, the supernumeraries do not pair in the female and because of their characteristic lagging are invariably lost, the progeny would receive their extra chromosomes solely from the fathers and thus would constitute only five classes, from zero to four, as follows: 70.19, 24.38, 4.75, 0.625, and 0.0625. Finally, if movement is at random in both sexes, progeny with up to eight supernumeraries should occur with the following frequencies: 49.26, 34.22, 12.61, 3.19, 0.63, 0.09, 0.01, 0.0008, and 0.00004 percent respectively, and in one further generation of random mating, with all classes equally viable, the zero group should decrease by slightly more than 0.25 percent.

Clearly, on the assumption of univalent loss, the frequency of extra chromosomes would be subject to pronounced reduction during succeeding generations—a rate of loss, in fact, that should ensure their virtual, if not complete, elimination over a period of 50 years or so. Judging by the frequencies observed at Chatham-East Lansing, either there is no sex differential in behavior at meiosis or there is selection favoring individuals carrying at least moderate dosages of supernumeraries.

The alternative to a cytological mechanism namely that the difference reflects a response to an ecological dissimilarity between Bryn Mawr some fifty years ago and Chatham-East Lansing in 1954, is at first thought rendered unattractive by the equivalent constitution of the Californian and Pennsylvanian populations in the early 1900's. Since, however, it is possible that changes in cultural practices, such as the widespread use of insecticides prevalent over the past several years, may have imposed new selective pressures that have made individuals with the higher numbers of supernumeraries adaptively superior, the latter alternative is not without a certain appeal.

Müntzing and his co-workers at the University of Lund have already established that the frequency of extra chromosomes in certain plant species is significantly different in diverse geographical regions, and they are now attempting to find out what ecological factors are possibly concerned. Extension of the present study over a number of years would doubtless prove profitable, but some doubt was experienced as to the wisdom of undertaking such a long-term study. My reluctance was occasioned by a number of considerations: the continent-wide distribution of the insect and the consequent necessity of imposing on other workers in securing representative samples for study, the difficulties encountered in shipping over long distances, the inroads made into local populations by control measures, and, most important, the near impossibility of obtaining information on the cytological constitution of females. A decision against continuing was, however, promptly reached when examination of a culture of *Tribolium madens* (Charp.), kindly donated by Dr. H. E. Gray, Entomology Division, Science Service, Ottawa, disclosed the presence in it of supernumerary chromosomes. The simplicity with which this flour beetle can be reared and its relatively fast development, together with the considerable general information already available on other species in the genus, qualify it as a logical choice to be preferred to *Diabrotica*.

Examination of *T. madens*, to date quite preliminary in nature, has sufficed to show that its supernumeraries differ from those of *Diabrotica* in, among other ways, having the ability to form bivalents in spermatocytes. Its

PRIMARY AND SECONDARY SPERMATOCYTES

Figure 6

← *A*—Metaphase I: 9_{II} + X + 4S (off plate). *B*—Metaphase I: 9_{II} + X + 4S (associated, at upper left). *C*—Metaphase I: 9_{II} + X + 4S + 1s. *E*—Two second metaphases: upper, 9 chromosomes; lower, 9 + X (spherical) + 1S (pale). *F*—Anaphase II: 2 divided S chromosomes.

neighboring species in Hinton's[2] synopsis, *T. castaneum* (Hbst.), has already been shown[6] to have 18 autosomes and an XY pair of sex chromosomes, in which the Y is relatively minute and associated with the X in the form of a parachute, and to differ from *T. confusum* Duval and *T. destructor* Uyttenb., both of which have two fewer autosomes and a neo-XY sex-determining mechanism. Cytologically, *T. madens* appears to be basically similar to *T. castaneum*, but the presence of supernumerary chromosomes having the capacity to pair confuses the picture, and a final decision must await the results of more extensive studies.

Dr. Gray's original stocks of *T. madens* came from two locations in Manitoba; examination of further material from distinct sources should provide information on the general distribution of supernumerary chromosomes within the species.

Summary

Examination of 100 males of *Diabrotica undecimpunctata howardi* from Chatham, Ontario, and 100 from East Lansing, Michigan, in 1954 established their supernumerary frequencies at 1.50 and 1.29 per male respectively, as compared with 0.67 at Bryn Mawr, Pennsylvania, in 1907. The supernumeraries are of two types: one larger and metacentric, the other about one-half the size and acro-or telocentic, both being positively heteopycnotic during early meiotic prophase. The smaller type constitutes 12.1 percent of the total—a 17-fold increase since 1907.

The supernumeraries move essentially at random and divide at either one of the two meiotic anaphases but not at both; hence no cytological mechanism exists in the male that would account for the numerical increase noted. The chromosome constitution of females could not be ascertained, but after consideration of the various ways in which their supernumeraries could behave, the increase is considered most likely to be due to new selective pressures making individuals with high numbers adaptively superior.

The occurrence of supernumerary chromosomes in a flour beetle, *Tribolium madens*, is recorded for the first time.

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UNDERDEVELOPED MANDIBLE IN A HERD OF DAIRY SHORTHORN CATTLE

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SEVERAL investigators have published lists of inherited lethal and sublethal characters in cattle. The number of characters varies from 25 to 30 in the papers consulted.[3, 4, 8, 9] Lerner[9] has given reference numbers to the lethal characters for farm animals—those for cattle being A1 to A25. This system of numbering is used in the present paper.

Cases of underdeveloped mandible, called impacted molars were recorded by Annett,[1] Heizer and Hervey,[6] and Ranstead[13] all in Milking Shorthorn cattle in New Zealand and the United States. Lerner[9] lists Annett's calves as agnathia A19 and those of Heizer and Hervey[6] as impacted molars A14. Ranstead[13] in his paper shows that the calves he reports and the calves reported by Annett[1] are from closely related stock and suggests that the latter's calves should have been listed by Lerner as A14. Annett did not examine as closely as the other investigators the deformed calves reported by him. In this abnormality the mandible is shorter than the maxilla and the pre-molar teeth are impacted. The character is inherited as a recessive. Snyder[14] refers to this abnormality as 'parrot-beak.'

Several cases of a similar abnormality called agnathia have been reported in Jersey cattle in the United States by Ely *et al.*,[2] A20, and in Ayshire cattle in Canada by Lalonde.[7] They differ from the Bonington calves as the lower jaws in the former appear to be much shorter or missing. In the cases reported by Ely *et al.* additional wattle-like appendages were present. Ljutikov[10] reported a dwarfed calf with underdeveloped lower jaw in Jaroslav Large Horned Cattle, a Swiss breed in Russia, A15. Gilmore[5] has observed similar, less acute, deformities which he calls brachygnathia in Holstein and Jersey cattle.

TYPICAL ABNORMAL CALF

Figure 7

The preserved head of calf "O" showing the short lower jaw typical of the abnormality.

Nordby *et al.*[12] record cases of brachygnathia in a flock of Rambouillet sheep. In this case what appeared to be a simple recessive inherited character was refuted when defective animals were mated. Too few offspring showing the abnormality were produced. The authors suggest this indicates the interaction of several pairs of genes.

Several cases of deformed calves were reported in the Bonington herd of pedigree and grading up Dairy Shorthorn cattle, belonging to the University of Nottingham. The herd, established in 1927, consisted of 100 milking cows and a similar number of young cattle divided between two farms. The breeding of the two herds of cattle was closely related, mainly by the exchange of bulls. The present investigation shows that 15 cases of underdeveloped mandible were recorded in the herds between August 1941 and November 1953. During this period an additional four congenitally

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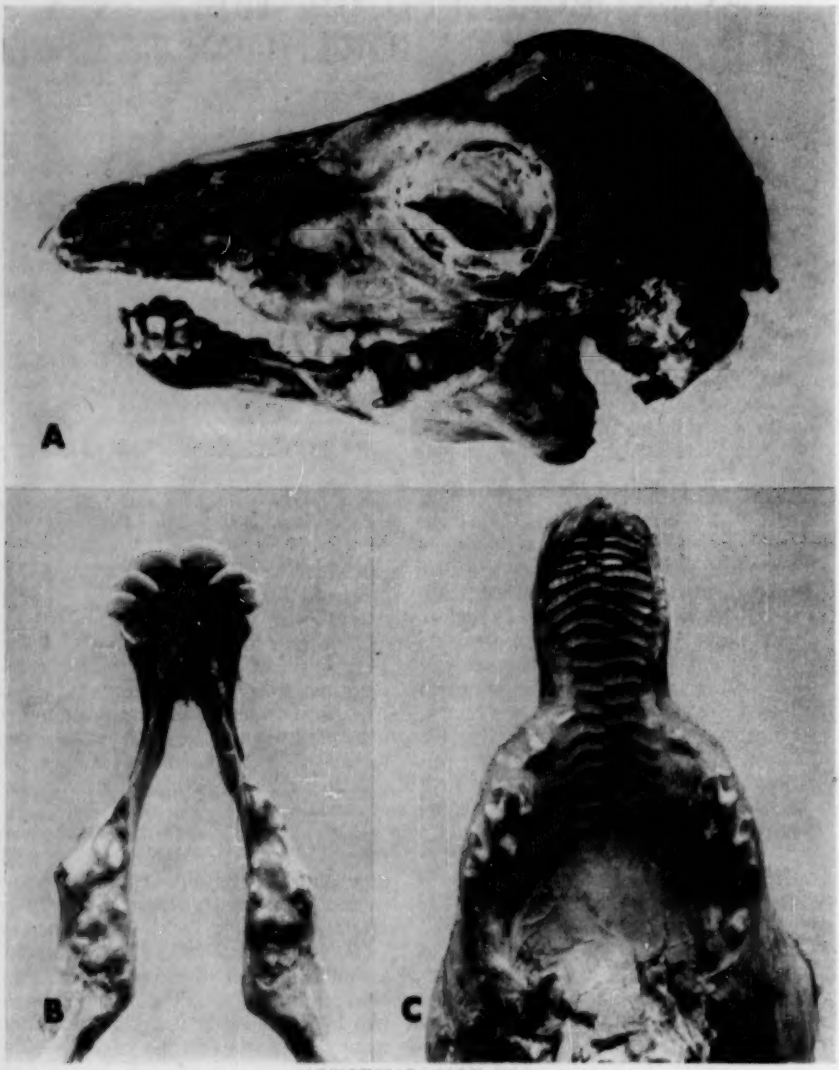


ABNORMAL CALF "O"

Figure 8

A—Shows a side view of the skull of calf "O". *B* shows the lower and *C* the upper jaws of calf "O".

abnormal calves were recorded. There is no description of the deformity in two cases, one was blind in both eyes and one was born prematurely with short forelegs (three inches long). In 1954 an abnormal calf was born to a cow at her 13th calving. In this case the calf had reflexed pasterns and its head was held in an upward direction, the animal was low in vitality and never stood up.

A number of hairless calves were also born prematurely but it would appear that the hairlessness was due to the earliness of the abortion and not to any hereditary factor for hairlessness.

Description

The head of calf "O" (Figure 7) was severed from its body after being humanely killed. The head was preserved in formalin and some 15 months later examined in detail.

The mandible was 2.5 cm. shorter than the maxilla before dissection and the tongue hung out of the mouth. When the head was dissected, after prolonged boiling in hypochlorite solution to soften the preserved flesh, the molars of both jaws were observed to be impacted and fused. The incisor teeth were normal (Figure 8).

The cases of underdeveloped mandible, six of which are recorded by Withers[16, 17] as undershot jaw, follow closely those reported by Annett[1], Heizer and Hervey[6] and Ranstead[13].

Incidence

The period covered by the investigation was 159 months and during this period 1202* calves were sired by 48† bulls. Seven bulls sired 315 calves of which 15 had an underdeveloped mandible (Table I).

Twelve cows produced the deformed calves. Ten of these cows had more than one calving but only one cow (No. 18) produced more than one abnormal calf. The twelve dams had 38 calvings of which one was premature.

*26 of the calves were crossbred Aberdeen-Angus or Hereford.
†3 of the bulls were Aberdeen-Angus or Hereford.

TABLE I. Analysis of the deformed calves under their sires

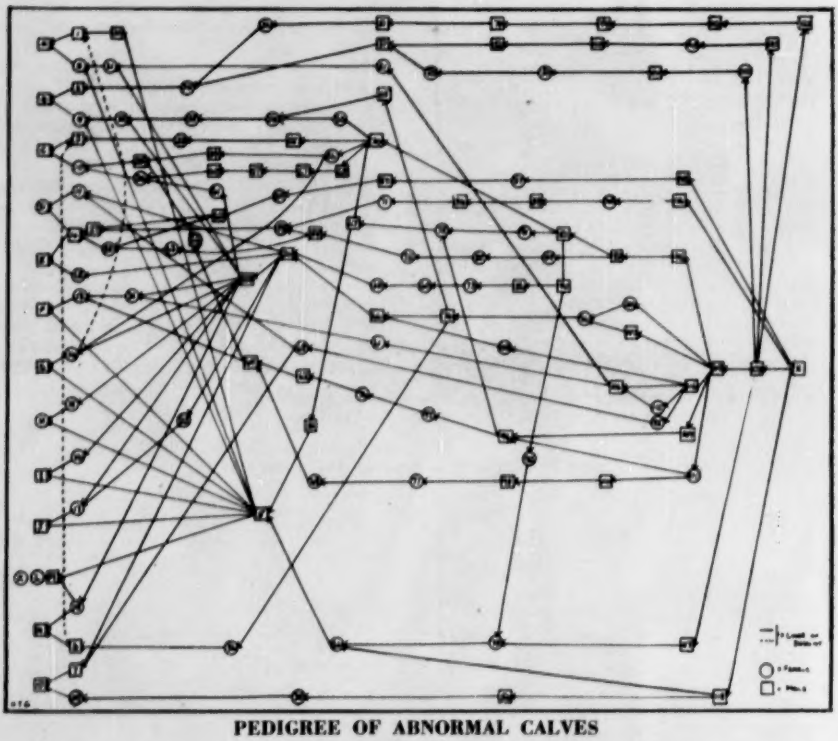
Sire	No. of deformed calves	Born alive	Born dead	Male	Female	No. of dams producing deformed calves	Total No. of calves out of dams tracing to "X"	Total no. of calves born to sire	Total no. of dams
1	1	1		1		1	12	12	12
2	1	?	?	1		1	19	10	10
3	1	1		1		1	8	8	8
4	2	2		1	1	2	54	109	91
5	8	5	3	5	3	6	87	108	80
6	1	1		1		1	36	39	36
7	1	1		1		1	17	29	29
Total	15	11*	3*	11	4	12†	224	315	216†

*One animal not recorded.
†Not a total of the columns; a dam may be recorded under more than one sire.

TABLE II. Analysis of the deformed calves under their dams

Dam	Total no. of calvings	No. of calving at which deformed calf was produced	Alive or dead	Male or female	Gestation periods Normal calves	Gestation periods Deformed calves
8	3	1	A	M	282 287	275
9	2	1	?	M	277	276
10	2	1	A	M	—	277
11	4	3	A	F	286 278 287	289
12	4	2	A	M	*	289
					296 285	
13	4	2	A	M	288 261 281	282
14	4	1	A	M	288 290 287	*
15	2	1	A	F	105†	276
16	3	3	D	M	*	281
					284	
17	1	1	D	M	—	290
18	8	3	D	M	*	283
		4	A	F	279	265
		5	A	F	280	282
		8	A	M	287	280
19	2	2	A	M	282	271
Total	38					

* Service date not recorded.
† Presumably aborted.



PEDIGREE OF ABNORMAL CALVES

Figure 9

The diagrammatic relationship of the abnormal calves is illustrated. All of the sires and all of the dams of abnormal calves trace to the common ancestor "X."

Where recorded the deformed calves were carried for full term gestation. Six of the calves were produced by cows calving their first calf, three by cows at the second calving, three by cows at the third calving, and one at each of the fourth, fifth and eighth calvings (Table II).

Nineteen successful matings took place between the seven bulls and 12 cows. Eleven of the calves showing the abnormality were male and four were female.

Relationship

All of the sires and all of the dams of abnormal calves trace to a common ancestor, a bull, hereafter referred to as "X" (Figure 9). This bull therefore occurs at least once on each side of the pedigree of all the calves with a short lower jaw.

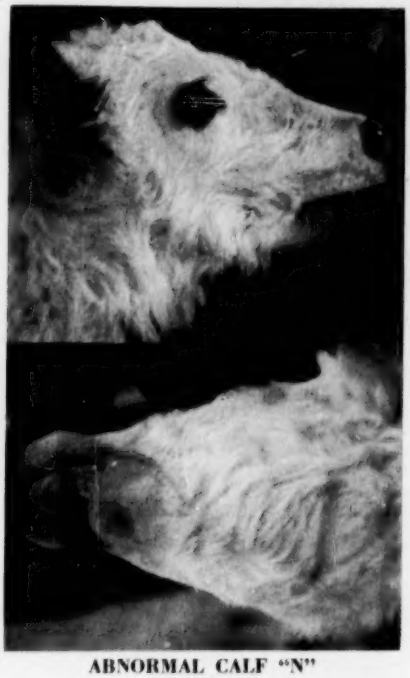
The pedigrees of the sires and the dams were separately traced to the sixth generation (seventh generation of the calves) and from the pedigrees traced thus far the relationship between the animals was established.

In the pedigrees of the deformed animals traced to the seventh generation, there is no apparent common ancestor except "X".

The coefficient of inbreeding of the calves calculated according to Lush[11] is small due to the remoteness of the relationship between the sires and dams. The largest coefficient of inbreeding is 9.4 percent for calf "A", that for the other calves being very much smaller.

Bull 5, a purchased animal, is the sire of eight calves (F, G, H, I, J, K, L and M), grandsire and great-grandsire of calf "A" and the great-great-grandsire of calf "B". No. 5 was several times a county show champion but was sold to the butcher when his daughters were found to be unsatisfactory for milk production.

Bull 29, a homebred animal out of purchased parents, is the grandsire of eight calves (G, H, I, J, K, L, M and N), and the great-grandsire of three calves (A, B and F) and the great-great-grandsire of "C". No. 29 was, after his early death from natural causes, a proven sire whose daughters yielded satisfactorily.



ABNORMAL CALF "N"
Figure 10

The profile of calf "N" is shown above. Shown below is a ventral view of the head of calf "N".

The sire (No. 7) of calf "O" and the dam (No. 11) of calf "D" are full sibs. Cow 14 produced with her first calving a blind calf "G" which had an underdeveloped mandible: her second calf was retained as a herd sire (No. 1). This bull sired a calf "A" showing the abnormality.

Calf "G" is the only animal with the short lower jaw recorded as being blind. It is possible that any of the stillborn abnormal calves would have been blind.

Bull "X" was twice a first prize winner at the Royal Show (England).

Discussion

The names given to this abnormality and similar conditions vary and the following list is derived from the various works consulted:

Agnathia	Parrot mouth	Fish face
Brachygnathia	Parrot beak	Fish jaw
Micrognathia	Overshot jaw	Pig jaw
Bird face	Undershot jaw	

No sire and only one dam (No. 18 which produced four abnormal calves out of eight calvings) was sold because they produced deformed calves. Only one of the sires was out of homebred parents and one was out of a purchased cow by a homebred bull. Four dams were out of purchased cows.

Wright[18] in his paper on monstrosities in guinea pigs refers to the production of abnormalities by environmental factors, that is mechanical, chemical, ultra violet rays, cold, etc. applied at an early stage in the development of the embryo. Williams [15] as quoted by Ely *et al.*[2] attributes the occurrence of monsters in dairy cattle to faulty methods of husbandry and also suggests that when disease involves the young foetus or an earlier stage in development and only partial recovery takes place, a monster may be produced. It would be difficult to trace the cause of the abnormalities in the calves under investigation to environmental agents. Between 1941 and 1953 inclusive, one calf showing underdevolped mandible appeared in each of five years, two in each of three years, four in one year and none in four years (Table III). No influence of season of the year was indicated in the incidence of abnormal calves. The monthly distribution of the abnormal calvings over the 13 years was similar to the distribution of the total calvings in the herd. Where a number of similar abnormalities appear among related animals over a considerable period of time, inheritance can reasonably be attributed the cause, or the predisposition to the agent causing the deformity may be inherited.

All the reports[1, 2, 6, 7, 10, 13] of impacted molars and agnathia in cattle classify the character as a recessive. In addition Ely *et al.*[2] finds agnathia confined to the male sex. Ranstead[13] found in a planned series of matings, that the abnormality occurred in the ratio of approximately one calf showing impacted molars to seven normal calves. He assumes the abnormality was introduced by a bull, Hippo (2944 N.Z.S.) who traces to ancestors imported from England, his grandsire being Ambush (87808 Coates Herd Book). Ranstead therefore reasons that the cases reported by Heizer and Hervey[6] probably inherited the factor from the same source as they were

TABLE III. Yearly distribution of abnormal calves 1941-1953. (Complete years)

Year	No. deformed calves	Total calvings
1941	1	65
1942	1	119
1943	1	107
1944	..	100
1945	..	90
1946	..	93
1947	4	89
1948	2	109
1949	2	94
1950	..	107
1951	1	92
1952	2	101
1953	1	97
Total	15	1,263

Milking Shorthorns of English origin. No relationship can be traced between Ambush and "X".

A large proportion of the animals which produce normal calves in the herd also trace to "X". Some indication of this proportion can be gained from reference to Table I. From a total of 315 calves sired by the seven bulls, 224 were from cows tracing to "X". In the whole herd during this period about seven-ninths of the cows passing through the herd trace to the bull "X".

The character is recessive and lethal, animals born alive being unable to suckle. Male calves appear to be more susceptible to the abnormality than female calves. The effect of a pleiotropic jaw-shortening gene could cause all the jaw abnormalities listed above.

No test matings were carried out and no attempt was made to keep an abnormal calf alive for more than a few days by pouring milk down its throat from a bottle.

Summary

Fifteen cases of underdeveloped mandible in Dairy Shorthorn cattle are reported. The abnormality is compared with similar charactors already recorded. The relationship between the affected animals is traced and the possible causes discussed.

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SOMATIC FLOWER COLOR MUTATIONS IN ALFALFA

KARLIS LESINS*

DURING the past few years, four different alfalfa plants have been observed in which purple or green flowers had lost their anthocyanin coloration, becoming yellow or white. These cases were investigated in order to get information on the nature of the mutations involved. In the course of studies some consideration was given also to basic problems regarding flower coloration and its inheritance in alfalfa.

Materials

The plant on which the flower-color mutation was first observed, and on which most of the work reported here has been done, was an artificial tetraploid F_2 plant. The F_1 plant ($2n = 32$) had been obtained by crossing a chromosome-doubled, originally diploid ($2n = 16$), *Medicago sativa* L. plant with a chromosome-doubled plant from a diploid ($2n = 16$) strain of *M. falcata* L. The F_1 plant had the flower color characteristic for hybrids: purple buds and very young flowers changing to green at full development and becoming greenish yellow in old petals. The mutant-bearing F_2 plant had the greenish flower color but of less intensity than the F_1. The mutant itself appeared as a branch on which flowers were yellow with no trace of anthocyanin coloration, neither in buds nor at a later stage of flowering (Figure 11*A*). Both the original and the mutant parts of the plant were propagated by stem cutttings; they are designated "219-53 green" and "219-53 yellow" respectively in subsequent discussion. During cultivation for three years there appeared once on the 219-53 yellow a small branch with greenish flowers, such as those on the 219-53 green, and once a part of a single, basically yellow flower was found to be greenish in color.

The second mutation was found in the progeny derived from the 219-53 green crossed to a white-flowered plant selected from cultivated alfalfa. Here again a branch with flowers of light yellow color, lacking any tinge of anthocyanin appeared on an otherwise greenish-flowered plant. In distinction to the first case, the flowers were lighter in shade both on non-mutated as well as on mutated plant parts. In subsequent discussion the original plant and the second mutation will be referred to as "No. 34 green" and "No. 34 yellow," respectively.

In the third instance, a single white flower was observed in a raceme of purple flowers (Figure 11*B*). This mutation, of course, could not be established as a separate plant. The mutation-bearing plant, hereafter denoted as "No. 3 purple," was a diploid ($2n = 16$) derived from an ivory-flowered F_2 plant of *M. sativa* × *M. falcata* crossed to a slightly pinkish-flowered plant of *M. hemicycla*. The diploid *M. sativa* and *M. falcata* strains were the same as for plant 219-53; the diploid ($2n = 16$) *M. hemicycla* Grossh, came originally from Transcaucasus.

In the fourth case, a yellow lengthwise band was detected in a green flower. The plant, on which the mutation occurred was a green-flowered hybrid denoted No. 240-1; it was derived from a haplo-Grimm plant ($2n = 16$) crossed to *M. falcata* ($2n = 16$). This cross has been previously reported.[8]

Regarding the application of the name "alfalfa" to the above material it may be noted that, though originally used for *M. sativa*, the name is now applied to the *M. sativa-falcata* complex. For example, several recently released alfalfa varieties have diploid, wild-growing *M. falcata* in their ancestry.

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A
B
C
D
E
F
G
H

Methods

Methods described earlier by the present author[9, 10] have been used in crossing, root cuttings, chromosome doubling and in cytological studies.

Histological investigations regarding location of coloring matter in petals were carried out under the microscope on free hand sections immersed in a solution of potassium and disodium phosphate adjusted to pH6.2.

In biochemical studies paper chromatography was used. Extracts of petals were obtained by using 1 percent HCl in water. For separation of anthocyanins a developing solvent consisting of 5 percent aqueous orthophosphoric acid was found very useful. Circular filter papers as described by Rutter[12] were commonly used for chromatograms of anthocyanins.

For studies of aglycones anthocyanins were separated on large sheets of filter paper using the ascending method. The separated anthocyanins were washed out with distilled water acidified with HCl (one drop concentrated HCl per 20 ml.). After hydrolysis in 2N-HCl for 20 minutes on boiling water bath, the freed anthocyanidins were taken up in iso-amyl alcohol and spotted on filter paper strips. Forestal[1] solvent was used for developing. As checks the following were used: cyanidin prepared from quercetin by the procedure given by Karrer[6]; pelargonidin, from flowers of light orange *Pelargonium*; malvidin, from violet red *Clarkia;* peonidin, from pink red *Petunia;* petunidin, from dark red *Petunia;* and delphinidin, from blue *Delphinium*. All checks were purified by paper chromatography and their Rf values compared with those given by Bate-Smith[1]

Morphological, Physiological and Histological Studies

Morphology and physiology. Plants grown from stem cuttings of 219-53 green and No. 34 green, and from their corresponding yellow mutants did not show any gross morphological differences. There were no differences in size or shape as compared to the purple sister flowers of the mutated white flower; nor in the yellow-colored lengthwise band as compared to the rest of the flower in the case of the fourth mutation.

When crossing and selfing were undertaken some differences between 219-53 green and its yellow mutant became apparent. In about 8 percent of the yellow flowers the pistils were so short that they remained enclosed in the staminal column (Figure 11C). Conspicuous differences were also found in the number of pods and in the seed set. At first it was thought that this was caused by non-effective pollination of the short-pistilled flowers. A pollination test, however, using the same number of normal pistilled flowers of the green and the yellow gave twice as many pods and nearly four times as many seeds on the green plant as on the yellow, viz., 42 pods with 85 seeds, and 21 pods with 24 seeds for the green and yellow plant respectively. Observation on pod development showed that about equal numbers of pods started to develop on both plants, but soon a portion of pods on the yellow plant became arrested in growth and dropped off. Obviously physiological disturbances present in the flowers of the yellow plant caused abscission of pods, and also abortion of embryos. The percentage of viable pollen grains (as indicated by staining) produced by the green plant was found to be 65 percent and by the yellow 63 percent. These values may be considered to indicate an equal degree of viability.

No clear cut differences were observed between No. 34 green and its yellow mutant regarding either pistils or fertility. Percent of viable pollen was found to be 38 percent in the green and 39 percent in the yellow flowers. Again the values may be considered equal.

Histological investigation. Histological examination revealed that the coloring materials are located in the epidermal layers of the petals. Cells of the inner epidermis (i.e., facing the staminal column) of the standard and wings are drawn to conical papillae (Figure 11*D*) and in these the pigments are mainly deposited. Cells of both epidermal layers of the keel are of a flat type, and the outer layer of the tip of the keel is usually the more intensely colored. In green flowers, the coloring material consists of yellow carotenoid plastids, located mainly at the inner walls of the epidermal papillae (Figure 11*D*) and of anthocyanins and yellow flavonoid pigments. The latter are of low coloring intensity, dissolved in cell sap and seen mainly in the papillae. In the yellow-mutant flowers the anthocyanins have disappeared with only the yellow coloring substances remaining.

Coloration of the green flowers may be understood by close examination of the epidermal layer. Not every epidermal cell has all groups of the coloring materials but some cells have yellow substances, some purple, and some both or none at all. Figure 11*E* shows patches of

FLOWERS AND CHROMOSOMES OF MUTANT ALFALFA
Figure 11

← *A*—Flower racemes of plant 219-53 green (right) and its yellow mutant (left). *B*—Purple-flowered raceme of plant No. 2 with white mutant flower in it. *C*—Staminal columns of flowers of 219-53 yellow with a normal (left) and a short pistil (right). *D*—Transection through a petal of 219-53 green showing epidermal papillae and yellow plastids (arrows) at the base of them. *E*—Epidermis of keel of 219-53 green with cells containing anthocyanin (dark) and cells without it (light). *F*—Meiotic metaphase I of 219-53 yellow with 1 I, 1-3 IV, and 9-13 II. *G* and *H*—Chromosome complements in root tips of 219-53 green (*G*) and yellow (*H*).

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1 2 3 4
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2

epidermal cells of the keel with anthocyanin coloration adjacent to cells containing only yellow color substances. Such a side-by-side, purple-and-yellow cell mosaic gives additively the perception of green color. Lighter and darker shades of green may be thought of as depending firstly on different amounts of coloring matter deposited in the cells. Secondly, cells containing both the yellow and the purple coloring substances will, when numerous, produce an effect of dark color. This color subtraction phenomenon and the role of papillae in flower coloration have been elucidated by Exners[2] and Frimmel[3]. Thus, the seemingly high saturation of color in flowers, as found on checking these against standards in color charts, is probably due to physical properties of the papillae.

Inheritance and Cytological Investigation

Inheritance. Inheritance studies were undertaken in order to obtain further information on the nature of these mutations. Some selfing was carried out with 219-53 green, No. 34 green and their corresponding yellows. Out-crossing was tried with 219-53 and No. 34, green and yellow on natural tetraploids, using an ivory-flowered plant designated 249-1 and two white-flowered ones designated R13 and R4-43-2. Results of these tests are presented in Table I. (In this Table and in Table II and III, flowers having anthocyanin constituents are referred to as purples and those without anthocyanin coloration as non-purples).

The breeding results in Table I indicate that there were not any significant differences between 219-53 green and its yellow derivative; nor between No. 34 green and its yellow part. Further, data on selfing and out-crossing to 249-1 indicate that one chromosome only was segregating and was responsible for anthocyanin production.

It was somewhat surprising at first to find that in crosses involving the two white-flowered plants only anthocyanin colored progeny was produced. The solution to this phenomenon was found by crossing the ivory 249-1 with the white R13 plant. All of the 12 plants raised from the cross contained anthocyanin in their flowers although the depth of coloration in different plants varied. Thus, complementary factors have been necessary for the production of anthocyanin coloration. The one factor, designated *A*, has been located in 249-1, the other, designated *B*, in plant R13. Plants 219-53 green and No. 34 green, and gamete producing tissues of their yellows should have had factor *B* in one, and factor *A* in two chromosomes—if preferential pairing of *Sativa* and *Falcata* chromosomes occurred—and *A* in at least three chromosomes if pairing had been at random. This latter question, on the type of chromosome pairing, was elucidated in crosses involving the strains of *M. sativa* and *M. falcata* used for the production of 219-53, as follows:

At the diploid level, several F_1 plants have on selfing given F_2 progenies totalling 108 purple to 33 non-purple colored plants. The F_1 backcrossed to *Falcata* have given 206 purples to 224 non-purples. In each of the separate families the ratios were in accordance with expectation. Thus, there was a clear-cut monofactorial segregation for anthocyanin production at random chromosome pairing. Similar results were recently reported by Twamley[15]. At the tetraploid level, one F_2 family consisting of 96 plants gave one yellow plant; in the other F_2 family, to which 219-53 belonged, there was one yellow out of 71 plants. The appearance of yellows decidedly excluded any assumption of a pronounced preferential chromosome pairing at the tetraploid level.

The most acceptable hypothesis which agrees also with data in Table I, seems to be random chromosome pairing. Factorial constitution of gamete producing tissues of 219-53 and No. 34, thus, may be assumed to be *AAAa Bbbb*; that of 249-1 *AAAa bbbb* and of R13 *aaaa BBBb* at the lowest factor dosage.

Cytology. Cytological investigations on root tips of cuttings revealed that instead of the expected 32 chromosomes there were only 31 in 219-53 green and yellow (Figure 11*G* and

TABLE I. Ratios of purple and non-purple flowered plants in progenies of greens, their yellow mutants, and of a relevant cross

Plant designation, and self or cross.	Number of plants with flower color		Fit to ratio
	Purple	Non-purple	
219-53 green, self	10	3	1:1
219-53 yellow, self	4	1	
No. 34 green, self	16	4	
No. 34 yellow, self	5	1	
219-53 green × 249-1, and recipr.	38	29	1:1
219-53 yellow × 249-1, and recipr.	18	18	
No. 34 green × 249-1, and recipr.	17	16	
No. 34 yellow × 249-1, and recipr.	9	6	
219-53 green × R13	57	0	1:0
219-53 yellow × R13	10	0	
219-53 yellow × R4-43-2	9	0	
249-1 × R13	12	0	1:0

MITOSIS AND CHROMATOGRAMS

Figure 12

A-C—Mitotic anaphase plates of plant No. 34 with disturbed chromosome segregation. *D*—A mitotic metaphase of plant No. 34 with two chromosomal bodies (arrow) in addition to the complement of $2n=31$ chromosomes. *E*—Chromatograms from flowers of 219-53 yellow (left semi-circle) and 219-53 green (right semi-circle), the latter with four different anthocyanins (semi-rings Nos. 1 to 4). *F*—Chromatograms showing five anthocyanins (rings Nos. 1 to 5) from flowers of progeny from cross ivory × white plant. *G*—Chromatograms of young (left semi-circle) and old (right semi-circle) flowers of an F_1 plant showing disappearance of No. 1 and weakening of anthocyanins Nos. 2 and 3 in old flowers.

H) as well as in No. 34 green and yellow. Obviously one set of homologous chromosomes contained only three instead of four chromosomes. It was of interest to investigate how the complement of three was transmitted to offspring and whether there was any connection between it and the purple flower color. Data for plants 219-53 green and yellow given in Table II have a bearing on this question.

Data in Table II indicates that there are no apparent differences between green and yellow either in transmission of chromosome number or in flower coloration. If the chromosome responsible for anthocyanin coloration had been one in the set of three homologues, then crosses with 249-1 should have yeilded purple flowered plants to non-purples in a ratio of 2:1 in the 32 chromosome group, and a ratio of 1:2 in the group of plants with 31 chromosomes. In the latter group the numbers found are significantly different from the ones expected, and in both groups the numbers of purples to non-purples are closer to the ratio of 1:1 expected on the basis of the anthocyanin-producing chromosome being one in a set of four homologues.

Further, these data show that in crosses with naturally tetraploid plants the gametes of 219-53 green and yellow with only 15 chromosomes have been functional as eggs as well as pollen. On selfing, however, there is a significant absence of 30-chromosome plants indicating that zygotes with only two homologous chromosomes of this particular set are not viable if other chromosomal sets are at the tetraploid level. This observation may have general validity in explaining the prevalence and stability of euploid chromosomal conditions commonly met with in plant species. Chromosome numbers other than those expected on the basis of segregation of the set of three are found in 8 percent of plants as seen in Table II. Aberrant chromosome numbers 28, 29, 34, and possibly 33 very likely originated in irregular distribution of chromosomes of sets other than the one containing three homologues. It should be noted that on another occasion[8] no irregularities of chromosome distribution were found in root tips of 63 artificial tetraploid plants examined.

The mother plant No. 34, bearing the branch with mutated yellow flowers, was transplanted to a greenhouse pot and root tip studies were made. It was found that out of 200 anaphase plates, 39 showed chromosome disturbances, (Figure 12*A-C*). The yellow mutant established by stem cuttings had 38 disturbed anaphases out of 208 cells examined. Consequently as high as 19 percent were found with irregular anaphase. One metaphase plate out of 63 counted showed, in addition to the complement of 31 chromosomes, what appeared to be two halves of two separate chromosomes (Figure 12*D*). Obviously, disturbances at chromosome separation have a bearing on initiation of the somatic mutations reported here.

Studies of meiosis did not provide much information. No clear interpretation of chromosomal configurations has been possible due to compactness and crowding of chromosomes. There were, however, always observed one or more configurations involving more than two chromosomes and, sometimes, a smaller body was seen which appeared to be a univalent (Figure 11*F*).

Biochemical Studies

Using the paper chromatography technique as outlined above, it has been shown that 219-53 yellow and No. 34 yellow have lost, in their petals, four anthocyanin compounds represented in Figure 12*E* by rings No. 1, 2, 3 and 4. Regarding compound No. 1 it should be noted that generally its intensity and visibility on paper chromatograms is strongly influenced by environmental conditions of plant growth, and by age of flowers. In extracts taken from flowers of plants grown under warm greenhouse conditions, especially in winter, and from old flowers, compound No. 1 was not detected (Figure 12*G*).

The other two mutant-bearing plants had one additional anthocyanin; this fifth anthocyanin as shown in Figure 12*F* by ring No. 5 was also present in progeny of ivory-flowered × white-flowered plants. In Table III data are summerized on the occurance of anthocyanins in mutant-bearing plants and in some relevant crosses.

From crosses given in Table III it may be seen that a factor, designated *M*, for anthocyanin No. 5 has been located in the white-flowered plant R13. Occurance in crosses 219-53 × R13 and 249-1 × R13 of plants with four and five anthocyanins and in the ratio indicated, is suggestive that factor *M* has been

TABLE II. Chromosome number in root tips of descendants of 219-53 green and yellow, and number of purple colored plants

Plant designation and cross or self	$2n = 32$ Number of plants		$2n = 31$ Number of plants		$2n$ = other chromosome no. (in parentheses) No. of plants
	Total	No. purple	Total	No. purple	
219-53 green × R13	4	4	4	4	0
219-53 yellow × R13	2	2	1	1	1 (34)
219-53 green × 249-1	4	2	4	2	2 (29, 33)
219-53 yellow × 249-1	4	2	5	2	1 (29)
249-1 × 219-53 green	2	1	8	7	0
249-1 × 219-53 yellow	6	4	2	0	1 (33)
219-53 green, self	3	2	10	7	1 (28)
219-53 yellow, self	0	–	5	4	0

carried by two homologous chromosomes of R13 and that chromosome segregation has been at random.

Some information was obtained regarding the aglyconic parts of the anthocyanins. After hydrolysis it was found that anthocyanin No. 5 had malvidin as its aglycone, anthocyanins Nos. 2 and 3 both had dephinidin, and No. 4 petunidin. Compound No. 1 because of the small amount available, could not be investigated regarding its parent substance. To judge from the position on chromatograms, No. 1 may be delphinidin without any sugar attached to it. It's prevalence on chromatograms from young flowers is similar to a case reported by Hayashi and Abe[4] where anthocyanidin was found in young flowers but was absent in old ones. Thus, the anthocyanins of alfalfa investigated so far represent the delphinidin series with delphinidin itself and its methylated derivatives, malvidin and petunidin.

The remarkable change of color taking place in hybrid flowers on aging from violet to dirty yellow could be clarified by comparing greens and their yellow mutants visually at different ages, and in addition using the chromatographic procedure. It was noted on visual inspection, especially on the palest mutant No. 34 yellow, that very young flowers were almost white, turning yellow later on. If the anthocyanin coloring material had been present in the young flowers it should have appeared as purple because of lack of additive and subtraction effects of yellow substances. This actually could be observed as the young flowers of non-mutated greens were purple in color. Taking the same number of very young and old flowers from an F_1 plant, using on them the same quantity of extracting fluid and spotting it in the same amounts on filter paper disks, it was shown that on aging of flowers some degradation of anthocyanins had taken place (Figure 12*G*). Consequently, change of color should be attributed to increase of the yellow substances and to deterioration of anthocyanins during the course of aging.

Discussion

When the first mutation was found it was thought that it might be a sectorial chimera. The normal 219-53 green, being an artificial tetraploid F_2 plant, might have lost the chromosome, carrying factors for anthocyanin color in a cell initial to a branch. This might have given the yellow mutant, its viability not being seriously impaired because of the tetraploid condition. Examination of pollen grains, however, indicated that such an assumption could not be valid because loss of one chromosome in the tissue which produced gametes should have given poorer rather than equally viable pollen in the yellow flowers. Later it became clear that, at least in cases 219-53 and No. 34 we were dealing with periclinal chimeras, the epidermal layer being mutant. Data on inheritance of flower color and transmission of chromosome number indicate that no differences exist between inner cell layers of greens and their corresponding yellows. Equal chromosome numbers found in root tips of cuttings from both mutated and non-mutated plant parts, also equal proportion of cells with disturbed anaphases in No. 34 green and in its yellow, were in agreement with the hypothesis of periclinal chimeras, as roots of stem cuttings develop from subepidermal layers and should be the same in the case of a periclinal chimera. Observation of a green branch, and a green part of a flower on 219-53 yellow may also be explained on the assumption that the inner, non-mutated tissue layers may sometimes break through the outer mutated layers and appear as back mutations. Histological finding that the site of coloring materials, especially anthocyanins, was the epidermal cell layers support the view that mutation cases 219-53 and No. 34 as well as No. 3 and 240-1 may be periclinal in nature.

There seems to be no difficulty in allocating differences in pistil length and in fertility of 219-53 green and its mutant into a common scheme. There are several known cases, compiled by Jones[5], in which periclinal chimeras have rendered male, female or both elements completely non-functional. A semi-inhibition of fertility and an incomplete penetrance of the short-pistil character as reported here is comprehensible and makes an addition to our knowledge on the many sided effects of periclinal chimeras.

It is highly credible that each of these mutations had its inception at somatic chromosome segregation as indicated by disturbed mitotic process in root tips of plant No. 34. It is likely that a cell which has lost a chromosomal segment, the one responsible for anthocyanin production, may give rise to an

TABLE III. Different anthocyanin compounds (indicated by Nos. 1 to 5) in flowers of mutant-bearing plants, and in flowers of plants of some pertinent crosses

Desig. of plants or crosses	Flower color	Anthocyanin compounds	No. of segregating plants tested
219-53	green	1, 2, 3, 4	–
No. 34	green	1, 2, 3, 4	–
No. 3	purple	1, 2, 3, 4, 5	–
240-1	green	1, 2, 3, 4, 5	–
219-53 × 249-1	green × ivory	1, 2, 3, 4	9
219-53 × R13	green × white	1, 2, 3, 4	4
		1, 2, 3, 4, 5	17
249-1 × R13	ivory × white	1, 2, 3, 4	1
		1, 2, 3, 4, 5	9

epidermal cell layer representing a anthocyanin-deficient mutation. Stickiness between chromosomes, often involving several pairs as shown in Figure 12*B* and *C*, may have given rise to segmental deficiencies. It may be noted that broken parts of chromosomes were observed (Figure 12*D*), but not deficiency or surplus of whole chromosomes. This may stress the probability that mutations have been initiated by somatic chromosome breakage and non-equal distribution to sister cells.

The fact that plants 219-53 and No. 34 both had 31 chromosomes suggests that the missing chromosome renders the process of segregation less stable for the remaining chromosomal complement not only at meiosis, as indicated in Table II, but also at somatic mitosis. It may be noted that in all cases mutations have occurred in hybrids in which the appearance or loss of anthocyanins may be confined to a single chromosome.

Most surprising was the finding that on all four different occasions, at each single mutational step, four or five anthocyanin compounds disappeared; and that, at a single step of complementary factor action, five anthocyanin compounds came into being. Chromatograms made of anthocyanin-containing flowers from nearly 150 plants, related to 219-53 by strain or closer relationship, have given without exception four or five anthocyanins. On the other hand, monofactorial mode of inheritance of purple flower coloration has been demonstrated. There remains but one inference, viz., that a number of anthocyanins behave as a single block. It may be reasoned that a precursor substance, produced by factor *A*, may be needed for the development of anthocyanins in *Sativa* and anthoxanthins in *Falcata* flowers. This would explain the monofactorial mode of inheritance as a rule observed in crosses between these two species. With complementary-factor action, as in the case 249- × R13, factor *A* would come together with factor *B*, this latter being a complex factor for the production of several anthocyanins from the precursor substance. In the production of mutations 219-53, No. 34, and 240-1 it may be postulated that factor *B* was removed from its chromosome while factor *A* and the anthoxathin-producing factor(s) remained, as indicated by color and histological studies of the yellow flowers. In the case of plant No. 3 it may have been factor *A* or *B* which was removed from a cell initial to a white-flower mutation. Whether this latter case is analogous to the one reported by Waldron[16] at which a white-flowered branch appeared on a lavender-flowered plant, cannot be determined.

A complex factor, like *B*, is not an exceptional instance in anthocyanin coloration. There are known cases in rice[11], maize[13] and cotton[14] where purple color of different plant organs has appeared to be inherited as a single factor, though on closer examination it has been found to consist of several pseudo-alleles. Our knowledge is too incomplete to explain exactly how complexes, like *B*, have come into existence. Lawrence *et al.*[7] have indicated that a factor for methylation, acting on a precursor substance related to delphinidin at incomplete methylation, may give a mixture of delphinidin, petunidin and malvidin. In our case there certainly is a separate step that leads to production of malvidin which is the aglycone of anthocyanin No. 5. It is likely that factor *M*, which is responsible for production of anthocyanin No. 5 is transferring part of an already existing anthocyanin to a higher methylation degree. This question, however, needs further investigation.

Further studies should also be made on the observed differences in intensity of flower color of plants 240-1, 219-53, and No. 34. All contain one dose of factor *B*, consequently, differences in color intensity should be attributed to dosage of factor *A*, to differences in genetical background, or to both. Also remaining for future clarification is the cause of the pink flush, observed in the cells of the outer epidermis of nearly white flowers of one of the parents which gave rise to the purple-flowered plant No. 3, this in turn giving the white flower. It is likely that factors other than those already mentioned will be discovered during further research.

Summary

Somatic flower color mutations involving loss of anthocyanin coloration were observed on four different alfalfa plants. In two cases inheritance of flower color and transmission of chromosome number indicated that the mutated branches were periclinal chimeras. In one of the cases loss of anthocyanins was associated with inhibited fertility and also with some undeveloped pistils.

Histological observations were in agreement with the assumption of periclinal chimeras since the coloring agents were found to be deposited in epidermal cells of the petals.

Cytological investigations on one of the mutant-bearing plants revealed that in about 19 percent of mitotic anaphase cells there were disturbed chromosome distributions. This supposedly may be the cause of the loss of a part of a chromosome, the one carrying a factor for anthocyanin production—in an initial cell giving rise to epidermal cell layer of the mutated branch.

Biochemical studies showed that in the process of mutations four or five anthocyanins were lost. The aglyconic portion of anthocyanins consisted of delphinidin and its derivatives. It is likely that in three of the mutational cases a complex factor *B*, responsible for the development of anthocyanins proper, has been lost; in the fourth instance *B* or a factor *A* needed for the production of a precursor substance, was not included in the mutated flower.

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PHOTOPERIODIC CONTROL OF ESTRUS IN THE BASENJI

JOHN L. FULLER*

THE Basenji, a relatively recent import from Africa, differs from most breeds of *Canis familiaris* in having an annual, seasonal period of estrus. According to Burns[2] the Basenji in Britain has changed from an annual cycle to the six-to-eight months cycle characteristic of domestic breeds. This phenomenon has not been noted in the colony of Basenjis maintained at the Jackson Laboratory. Here estrus has been concentrated in the fall with the peak (60 percent) falling between September 20 and October 10. The mean date of beginning estrus was October 6 in a sample of 49 periods observed in 24 females. The frequency distribution is shown in the middle portion of Figure 13. One aberrant estrus period was observed in January in a female which had whelped six weeks previously, but who had not raised the litter. This female was receptive to the male, but failed to conceive. The case is omitted from the figure, and from the computations, since it is believed to represent some unusual but unknown set of circumstances. In contrast the distribution of the beginning dates of 103 estrus periods observed in 34 Cocker Spaniel females maintained under the same conditions shows only irregular variations throughout the year. The monthly frequencies do not differ significantly by the chi-square test.

The regularity of the onset of estrus in the Basenji suggested the hypothesis that the cycle was synchronized with the annual cycle of changing length of the daylight period. Photoperiodic control of the reproductive cycle in vertebrates was reviewed a number of years ago by Bissonnette.[1] Many species of birds, a few mammals and some insects were listed as responding to increased day length by increased gonadal activity; a few species responded in a similar manner to shortened days; while still other species were unresponsive to changes in this variable. Most of the forms dealt with were wild species, although the guinea pig

*Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The author is grateful for the assistance of Mr. Albert Pawlowski who tabulated the data on dates of estrus for the control samples.

was included with the non-photoperiodically controlled group. Dawson[3] accelerated gonadal development and produced early estrus in laboratory cats by increasing the daily duration of illumination in the cat colony room. No mention has been found of racial differences in photoperiodic sensitivity of the reproductive cycle within a single mammalian species. Hooyer[4] speaks of the possibility of such differences in the races of *Salmo irideus*, but gives no data.

Experimental Results

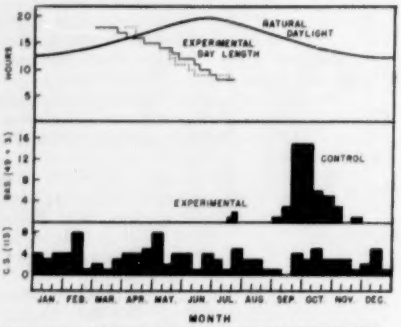
In order to test the hypothesis that estrus in Basenjis is precipitated by exposure to a period of declining day length, experiments on three purebred females were carried out during the springs and summers of 1952 and 1955. Subjects were kept in heated quarters which could be darkened. The daily light cycle was controlled by an electric clock and switch device connected to a 75 watt incandescent lamp. The solid line in Figure 13 represents the artificial day length in 1952; the dotted line, 1955. The smooth curve gives the number of hours of daylight (twilight through twilight) for Boston.[5] Since Bar Harbor is north of Boston, and since the control animals were maintained indoors where the effective hours of daylight would be reduced, this curve must be considered as only an approximation to the amount of light received by the controls.

The dates of beginning estrus for the three subjects were July 15, 1952, July 25, 1955 and July 28, 1955. The latest date is 8.75 σ from the mean date for the controls, so that despite the small number of cases the statistical significance of the results is extremely high. The onset of estrus followed the start of the gradual light reduction by 111, 104 and 107 days in the three cases. The majority of the control dates fell between 80 and 100 days after the summer solstice. The design of this experiment does not permit any conclusions regarding this difference in the rate of inducing estrus.

Each of the experimentally induced heat periods was characterized by the usual physical and behavioral signs, and in each case a successful mating was obtained and normal puppies produced.

It may be concluded that the estrus cycle of the Basenji is synchronized with the natural light cycle. Natural changes in day length have no major influence upon the estrus cycles of Cocker Spaniels. Experiments on Basenji-Cocker Spaniel hybrids are now in progress in an attempt to determine the mode of inheritance of the differential physiological response to light. The F_1 seems to be non-photoperiodic, though complete data is not yet available.

A difference in neuro-hypophyseal relationships is the most probable explanation of the difference in reproductive periodicity which



LIGHT CONTROL AND FREQUENCIES
Figure 13

The upper portion of the graph shows the hours of light for experimental Basenjis, and day length for controls. In the center is shown the frequency of dates of beginning estrus in 24 female Basenjis. The lower portion shows the frequency of dates of beginning estrus in 34 female Cocker Spaniels.

separates Basenjis from other dogs. Since the functional variant is under genetic control, it could be utilized in endocrinological research.

Several explanations may be offered for the adaptive change in the reproductive periodicity of British Basenjis and the conservatism of Maine Basenjis aside from the hypothesis that this reflects the political temperament in the two regions. Genetic variability may have been present in the original African imports, and the non-seasonal breeders may have been selected in Great Britain. Another possibility is that the extreme day length changes at more northerly latitudes have somehow disrupted the coordination of light and reproductive cycles by overstraining the mechanism. Intrinsic factors may then govern the rate of physiological change independently of light. If this is true, annual periodicity could be restored by controlling the cycle of day length.

According to breeders, some American Basenjis have broken away from fall breeding. In one case brought to the author's attention, a Basenji bitch was bred in April. It is of interest that this animal was kept indoors part of the time under conditions which unwittingly paralleled the experiments reported here.

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A DESCRIPTION OF POLYDACTYLISM IN MICE

RICHARD J. ENGEL*

THE word polydactylism comes from two Greek words which mean much or many and finger or toe.[10] As used in biology, it indicates many or several extra digits beyond the normal number for any species. It is generally found occurring either on the hands or on the feet or on both and is sometimes associated with peculiar teeth defects. Polydactylism is found in several species including man.

It is known that polydactylism occurs on the hind feet of mice. The abnormality favors the right foot. However, a double or bilateral manifestation is frequent. The first generation of offspring of a cross between mice of a polydactylous strain and mice of another strain never show any signs of the abnormality according to some authors. From the F_2 generation, and usually thereafter, the number of mice born with the abnormal condition increases with selection. However, such things as maternal age[8] and environment play an important part as to the number of mice that are born with a polydactylous condition.

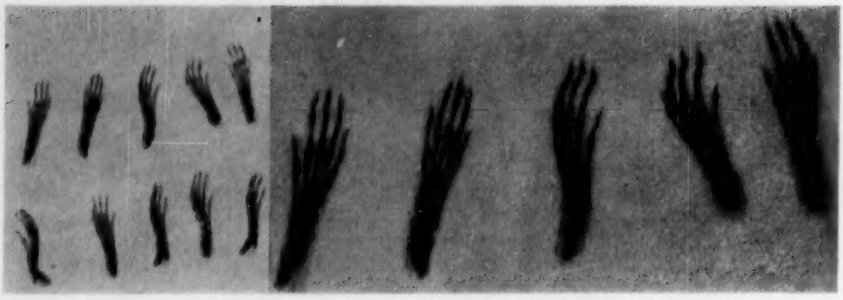
It is the intention of this paper to bring together the views of many qualified persons who have studied in this field, and along with this author's findings, present a clear description of this abnormality in general. The main purpose however in the present paper is to make a fairly complete description of polydactylism in a strain of mice now housed in Springville.

Certain sources show that polydactylism is due to a single recessive gene. The degree of the manifestation is affected by certain modifying factors, which are also inherited, hence the increase in successive generations by selection. A type of polydactylism was described by Chase[2] in which matings of polydactylia by polydactylia produced a frequency of expression of about 25 percent. His data from reciprocal outcrosses and subsequent matings indicate a single sex-linked recessive gene as the simplest explanation. According to Chase, this is the first case of sex-linked inheritance in the mouse.

In a study made by A. B. D. Fortuyn[6], the first polydactylous mouse in his strain belonged to the 27th generation of inbreeding. According to Fortuyn, this "indicates that somehow the environment is responsible." More of the young males of Fortuyn's strain survived to the age of one month than did the young females of this same strain. However, there were more females that had the abnormal condition. The author also stated, "in litters of normal mothers and abnormal fathers, the difference between abnormal daughters and abnormal sons became 18.58 ± 5.16 percent. Therefore, it is justified to say that abnormal fathers produce more abnormal daughters than abnormal sons."

The various authors working with this morphological characteristic designated *py* as the gene responsible for polydactylia in mice. Fisher[4], writes that homozygotes of *py* are not always polydactylous, as would be expected by the inheritance of a single recessive gene but may have normal feet. He states, "A point of evolutionary interest is that a morphologically similar mutation is known in several species of birds, and there it is found to be practically dominant, or to put it more accurately, not completely recessive. In mice the mutant is not only completely recessive, but appears to have advanced very far in the succession of changes needed to suppress

*Griffith Institute, Springville, New York. Acknowledgment is due to Dr. Leonell C. Strong, Director of the Biological Station of the Roswell Park Memorial Institute of Springville, New York, for his kindness in suggesting the present problem and supplying the materials for its investigation. He has also very generously aided in criticism of the manuscript. The Radiation laboratory of the Roswell Park Memorial Institute did the X-ray work.



NORMAL FEET

Figure 14

An X-ray photograph of normal hind feet of mice is shown at two magnifications.

its actions even in the homozygote."[4]

Murray[9] mentions that polydactylism has been studied in humans, guinea-pigs, and poultry. He concludes that, "From these observations it is regarded as a dominant character, controlled by certain unknown modifying factors." He observed that mice in certain inbred strains underwent a mutation in hair color from chocolate brown to leaden. At the time Murray wrote, only one polydactylous mouse had been observed in this stock. However, to conclude from a single case that the condition is dominant is perhaps not justified.

Foster[5] in discussing Wright's original work tells of a study that showed a marked depression of the number of young polydactylous individuals that were born before the female guinea-pig had reached maturity. He questioned if there would be competition between the mother and her offspring for some substance, or if it could be due to hormone changes in the female during development to maturity.

In a pedigree of a German family published by Windie,[3] a normal woman and a man with polydactylia had two children with polydactylia. This suggests a possibility of the inheritance of a dominant gene.

In a personal interview with Dr. L. C. Strong, of the Roswell Park Memorial Institute, I was told that polydactylism is believed by some investigators to have some similarity in inheritance to certain types of cancer in mice. These two conditions are similar in the fact that apparently the age of the mother at the time the offspring are born influences the expression of the character. In this same class may be placed Mongolian idiocy or Mongolism in man. By working with mice and other animals, the mode of inheritance of polydactylism and cancer may be solved.

Material and Methods

There is a strain of polydactylous mice at the State Biological Station at Springville, New York. This is a brown belt subline (symbol *Brbelt*) of the NHO strain. These strains originate in New Haven, Connecticut, and were brought to Springville in the fall of 1953.

As the mice with the polydactylous condition matured (between one to six months) and were killed, the hind feet upon which the abnormality always appeared were amputated and put in 70 percent alcohol for preservation. When several hundred cases had been accumulated, they were examined and divided into eight separate classes. These classes were based upon the degree of manifestation of the condition. A control group of normal mice was also prepared in the same way for comparative study (Figure 14).

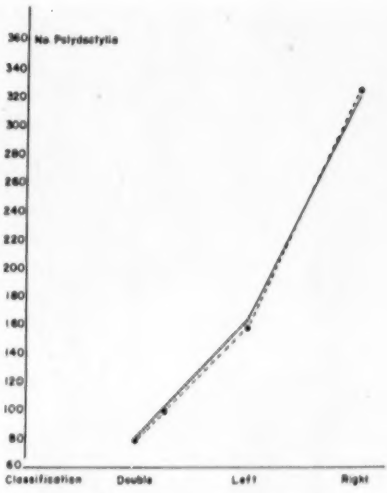
The types were named from Type I to Type VIII as follows:

Type I—Thick first or great toe (hallux).
Type II—Thick first toe with partial split near apex.
Type III—Six toes with incomplete separation (double hallux).
Type IV—Six toes with complete separation and outer extra digit longer than medial toe.
Type V—Triplication of the first toe.

Type VI—Outward growth of extra digit.
Type VII—Extra digit extra long.
Type VIII—Webbing (syndactylia) and miscellaneous variations.

The feet were also divided into groups of right and left. Some of the best specimens were taken to the Roswell Park Memorial Institute in Buffalo, New York, and X-rayed according to their specific classifications (Figures 14, 16, 18, 19). The X-ray plates were numbered so that an accurate accounting could be made of each X-ray. Then a study of the incidence of recorded polydactylism at the State Biological Station was made. Polydactylism is readily observed at birth; therefore, as each litter of the polydactylous strain of mice is born, the number of polydactylia born is recorded.

Special emphasis was given to sex, which foot was afflicted, and cases of a double or triple manifestation. These classifications concerned the mice of the F_1 to F_7 generation, following a cross between mice of the polydactylous strain and another strain in which polydactylism has never been seen.



NUMBER OF POLYDACTYLOUS MICE

Figure 15

This chart shows the number of polydactylous mice classified according to distribution of polydactylia on left and right hind feet and on both hind feet.

Results

In a total of 309 amputated polydactylous hind feet observed, 202 were right feet and 107 were left feet. This suggests an approximate ratio of 2:1. On a 2:1 ratio basis one would expect 206 rights to 103 lefts. Thus polydactylia occurs twice as frequently on the right foot as it does on the left.

In a total of 578 observations 159 mice had an abnormal or polydactylous left foot, while 334 mice had a manifestation of the right foot. A total of 81 mice had polydactylism on both hind feet. The ratio observed, 81:159:334 is very near what one would expect on a 1:2:4 basis which would be 82:164:338. There were also four mice that had triplications on the right foot (triple hallux). In all cases the female had a greater majority of the extra toes. This excess is more than what would be expected by a slight excess of females over males in the population.

Type I was of rare occurrence. The classification consisted of thick great toes which did not show on the surface any signs of doubling. However, once these toes were X-rayed, they showed the double row of phalangeal bones. These toes merely appeared thicker than usual and would not be associated with polydactylia if it were not for the X-rays.

Type II was the largest group. It was the class which had the abnormal toe which was thicker than usual with some indication of a start of an extra digit. For instance, the usual first toe was thicker than a regular toe and might have had two sets of nails. When these toes were X-rayed the X-rays showed a double column of phalangeal bones. On the surface, the hallux appeared to be Y shaped (Figure 16*B*).

Type III was a type of foot with six toes which had an incomplete separation. No claw had as yet started to form. These were apparently a further development of Type II (Figure 18*A*).

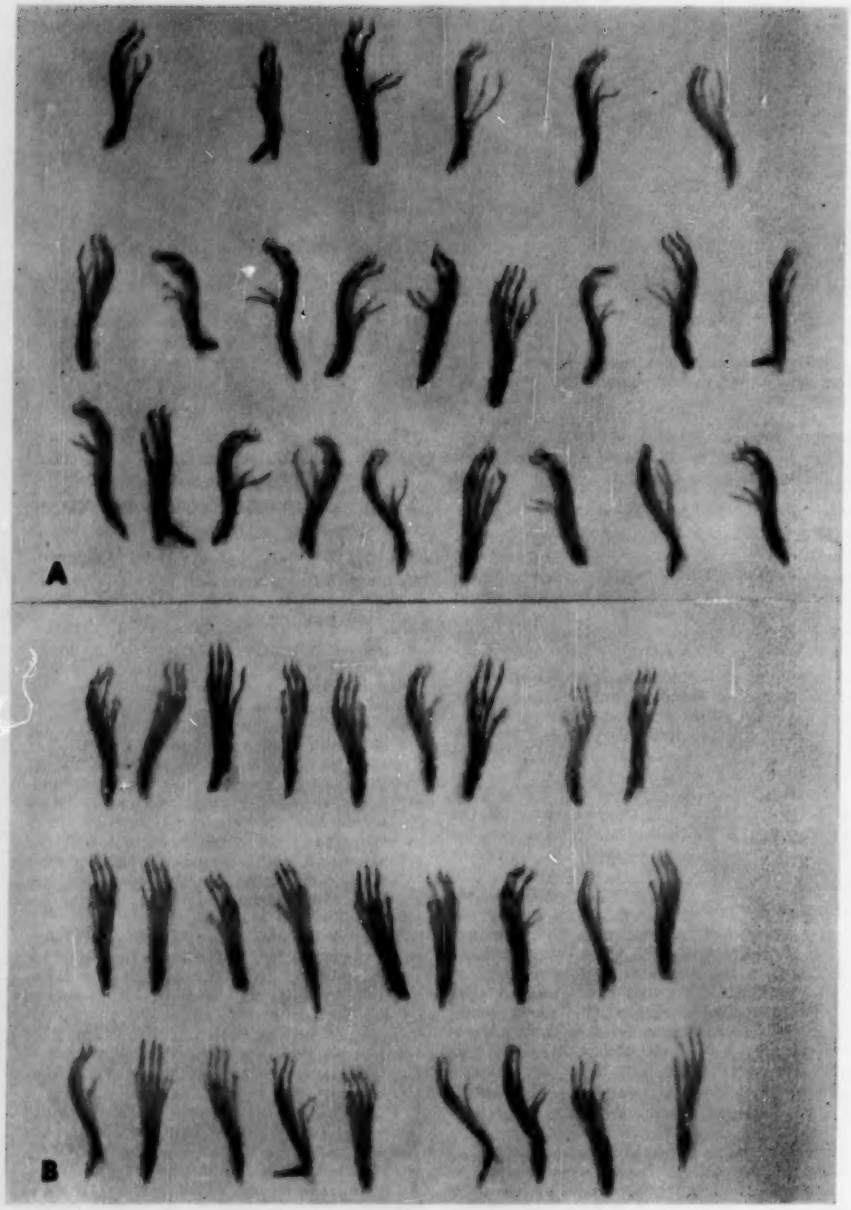
Type IV was the second largest class. It consisted of feet which had six toes with complete separation. The extra toe was found to be structurally different from the other toes in many cases. In some cases, the first toe and the extra digit formed a type of double claw. In other cases, the abnormal toe had grown unusually long and was as long as the other toes of the foot (Figure 18*B*).

Type V was made up of seven toes. These were always a triplication of the first toe (triple hallux). In all three cases, the manifestation of seven toes had not yet completed its full development (Figure 19*C*).

Type VI was quite distinct from the other types of abnormality. The extra digit grew at right angles away from the first toe. Also, the extra toe was not very long (Figure 19*A*).

Type VII consists of very long extra digits. This type formed a claw between the first toe and the extra digit.

As may be seen in the photographs, Types I, II, III, IV, V and VII have one feature in common. They all lead to, or possess the development of a sixth toe in which the extra digit grows in the same direction as do the rest of the toes on the feet. Type VI is the



TWO TYPES OF POLYDACTYLIA

Figure 16

An X-ray photograph shows types I and II of polydactylia; (double Hallux).

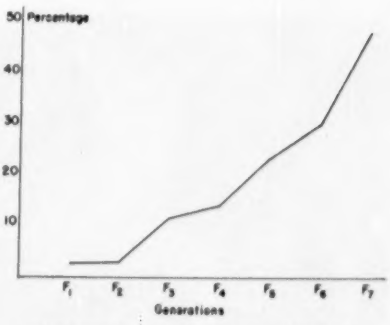
only case where the extra toe grows in another direction and is shorter (Figure 19*A*)

In Type VIII another new feature makes its appearance. An example of syndactylia is present. A webbing occurs between the first toe and the extra digit so as to give the appearance of a webbed claw (Figure 18*C*).

In the greatest majority of cases observed, the first toe of the right hind feet was most frequently affected. When the abnormality is slight, a thickening of the first toe results. This is followed by a stage of growth when the toe is provided with an extra nail. The thickened toe also has two columns of phalangeal bones. First the nails split and finally the bones separate and a sixth toe begins to develop. When the series of duplications have ended, the extra digit is on the outer side of the foot and is larger than the first toe. The two toes then form somewhat of a claw.

Normally the first toes of mice have two phalanges while the other toes have three. The formula is 2:3:3:3:3, while in mice with polydactylism the formula is 3:2:3:3:3:3. The extra digit has more phalanges than the normal first toe, except in Type VI. The feet look as if they normally had six toes. In the most extreme cases. the duplication may again develop on the same hallux and seven toes will be present. In this study the classification of amputed feet, Types I-V were believed to be different stages of development. Therefore, the actual number in each class would not be important, because the mice were killed at different ages. Some of the feet were fully developed while others were not.

In reviewing the records at the State Biological Station, the following information was found and then made into a graph (Figure 17). In the F_1 and F_2 generations the number of polydactylia born in the *Brbelt* strain was about 3 percent. In the F_3 generation it was 11 percent, in the F_4 generation it was 14 percent, in the F_5 generation the percentage was 24 percent. A 30 percent manifestation was reached in the F_6 and 48 percent manifestation was obtained by selection of polydactylia by polydactylia in the F_7. Apparently the number of polydactylia begins to increase sharply at about the F_4 or F_5 generation. The line graph then rises rapidly but there are no data suitable for determination of any more points beyond the F_7.



INCIDENCE OF POLYDACTYLIA

Figure 17

This chart shows the increase in the percentage incidence of polydactylous offspring in succeeding generations. In the F_5 generation and onward only polydactylia × polydactylia are included.

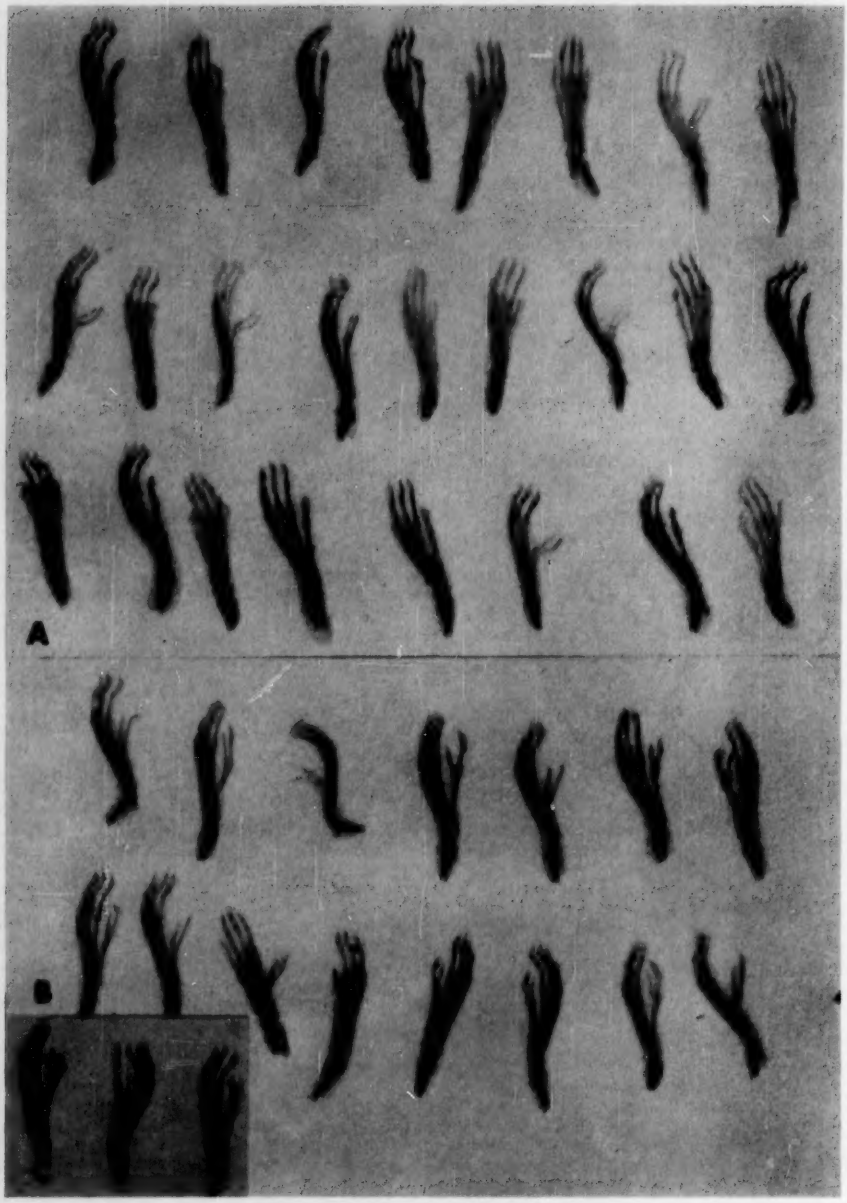
Discussion

All of the eight types of polydactylia described in this study were similar to the manifestations discussed by Fortuyn[6] and various other authors summarized by Grüneberg.[7] In significance to anatomy, there appears to be three regions on the developing hallux where the extra digit might originate. One region is at the base between the first and second toes; the second region is at the top or tip of the hallux; the third region is at approximately the lower portion on the outward aspect of the hallux probably near the region of its attachment to a metacarpal. However, there is the possibility that there is no definite or set region on the hallux for the development of the extra digit because the condition of double or triple hallux varies to a great degree. This point could only be determined by serial sections of the embryo, which were not prepared in the present study.

Genetically speaking, the polydactylous condition in the strain of mice observed (*Brbelt*) appears to be incompletely recessive in character and controlled to a great extent by certain modifying factors which are evidenced by selection between the F_1 to the F_7. In addition, however, such influences as maternal age and environment also play a role in the development of the character. Fisher[4] writes that a powerful suppressor has been found by which a single gene substitution seems to reduce the frequency of polydactylia about tenfold. The modifiers of the gene vary so, it makes it very hard to set a pattern of inheritance. According to Phillips,[11] the inheritance does not follow a simple Mendelian pattern.

Tsang[12] investigated the possibility of overdeveloped number of motor horn cells on the affected side of polydactylia and concluded that they might be the cause underlying the formation of extra toes in his strain. However, Chang[1] investigated the same strain of mice and did not agree with the conclusion of Tsang.

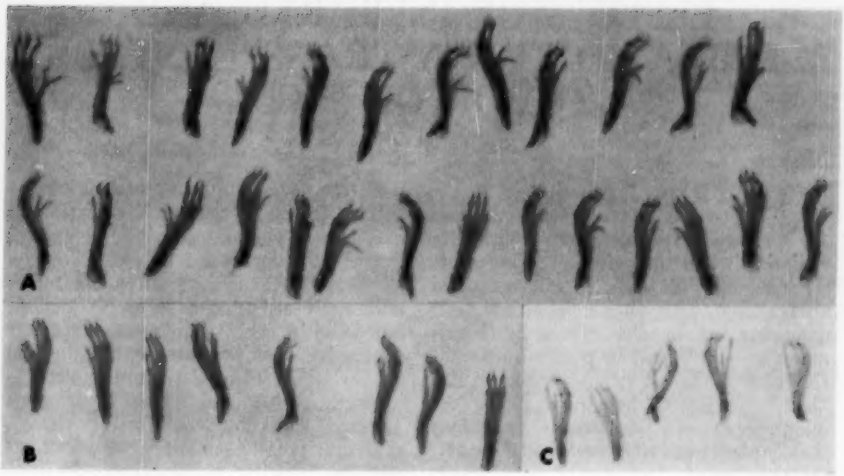
I have mentioned that the abnormality is readily observed at birth and the number of polydactylia in each litter can be recorded immediately. This supports the theory that the



TYPES III, IV AND VIII

Figure 18

A, *B* and *C* shows respectively the types of polydactylia designated III, IV and VIII.



TYPES V, VI AND VII

Figure 19

A represents type VI, *B* represents type VII and *C* represents type V of the polydactylous anomaly.

manifestation begins in the early embryonic life of the mouse (prepartum).

Conclusions

The *Brbelt* strain of mice possesses an inherited mechanism for the appearance of polydactylia that is affected by certain modifying factors.

A new ratio—1:2:4—has been established that shows the relation of the manifestation between bilateral cases: left cases: right cases. As far as could be ascertained, this conclusion has never been reached before by other authors in this field of study.

The polydactylous condition starts in the embryonic stage of the mouse and continues until the mouse has developed fully.

There appear to be three foci on the hallux which may be involved in the development of the extra digit (polydactylia) all of which are in the plane of the entire foot.

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EFFECTS OF INFRARED AND X-RADIATION ON A TRANSPLANTABLE TUMOR OF MICE

GORDON M. CLARK*

NEAR infrared radiation is an effective potentiating agent when used in combination with X-radiation when the criterion of effect is the frequency of induced chromosomal aberrations. This phenomenon has been demonstrated in *Drosophila* by Kaufmann *et al.*[11,12] and in plants by Swanson and Hollaender,[18] Swanson,[19] and Yost.[21,22] Beatty has observed in the microspores of *Tradescantia paludosa* that under certain experimental conditions, infrared alone has a lethal effect on some cells in division.[2]

X-radiation is known to kill tumors of mice *in vitro*;[17,13,10] and *in vivo*.[15,20]

Melnick and Bachem[14] studied the effects of X-radiation on tumors in rats and showed that for fast growing tumors a saturation technique was the logical choice. This saturation technique consisted of a high initial dose which destroyed the sensitive cells followed by subsequent doses which destroyed the refractory cells. There seems to be general agreement that the use of repeated small doses of radiation at selected intervals is more effective for some tumors than more destructive doses given less frequently or than single maximum tolerated doses.[6]

Rhodenburg and Prime[16] treated mouse and rat tumors with diathermy combined with X-radiation and reported regressions in tumors which when treated with either diathermy or X-radiation alone showed little inhibition. Fuchs[9] reported beneficial effects on breast tumors in humans by combining shortwave pre-treatment with post-treatment by X-radiation. He found an increase in sensitivity to the effects of X-radiation in the tumor tissue. Bonét-Maury and Patti,[3] however, found no effects on the survival time of mice after treatment with an X-radiation dose of 1000r combined with such agents as six-meter shortwave therapy, intense illumination or darkness, infrared or ultraviolet radiation. Beatty and Clark report that infrared radiation combined with X-radiation greatly enhances the effectiveness of X-radiation on tumors in mice when the criterion of effect is the percentage of tumor regressions attained. Both infrared and fractionated X-radiation when used singly effect tumor regressions. Single massive doses of X-radiation were ineffective against the same tumor at the dosages employed.[1]

This paper reports a study of fractionated X-radiation, fractionated infrared radiation, and the effects of various exposures of fractionated infrared radiation combined with a constant dosage of X-radiation on a transplantable mammary tumor in mice.

Materials and Methods

A rapidly growing mammary carcinoma, F191, was used in these studies. This tumor has a latent period of three to five days, takes in 100 percent of the animals and kills the host in three to five weeks. Spontaneous regressions have never been seen to occur, necrosis is not extensive and the tumor metastasizes primarily to lung tissue. Week-old transplants were excised and cut into small pieces. These were transferred by means of a glass pipette through an incision in the inguinal region into the right axillary region of the animal. The transplants were allowed to grow

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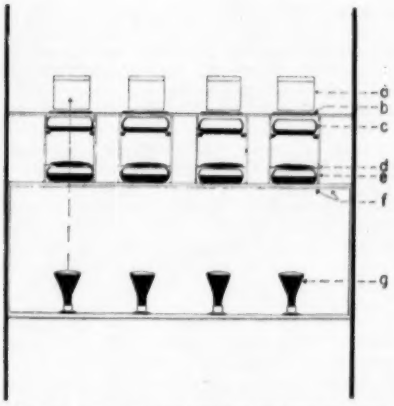


INFRARED RADIATION EQUIPMENT

Figure 20

a—animal cage, *b*—sheet of glass, *c*—pyrex flask containing iodine-saturated carbontetrachloride solution, *d*—condensing lens, *e*—pyrex flask through which a continuous supply of cool water circulates, *f*—asbestos sheeting to protect wooden frame from heat, *g*—infrared lamps. The arrow indicates the direction of the passage of infrared rays through to the caged animals.

from one week to 10 days in the new host before being used in the experiment. Tumors at this time averaged 1.8 cm in length by 1.0 to 1.5 cm in diameter. Rockland Farm virgin Swiss female mice weighing 18 to 25 grams were used as hosts for the transplants. The animals were maintained on Purina Laboratory chow and water *ad libitum*.

The infrared was obtained from 250-watt General Electric infrared industrial type reflector lamps operated at 110 volts. These were placed 17 inches from the caged animals. Interposed between the lamps and the caged animals, at a distance of nine inches above the bulbs, was placed a flat pyrex flask (1000 cc capacity) through which a continuous supply of cool water circulated. The depth of water in the flask was maintained at two cm. Directly above the water filter was placed a biconvex condensing lens five inches in diameter. Rays passing through the water filter were focused by the lens on and through a second pyrex flask containing a 1.0 cm. thickness of a solution of iodine-saturated carbon tetrachloride. This latter filter was located 13 inches above the infrared lamps and four inches from the caged animals. The cages measuring six inches in length, 5½ inches in height, and 5½ inches in width rested on sheets of glass 3/16 of an inch thick and measuring 8½ by 11 inches (Figure 20). All animals (both treated and control) were housed five to a cage. This apparatus eliminated most of the infrared radiation above 14,000 A.°

Infrared radiation was given as an initial pre-treatment of 24 hours' exposure with varying exposures at 24-hour intervals thereafter, for total treatment times of 36, 48, 60, 72, 84, 96, and 108 hours (See Table I for exposure times). Twenty animals were used for each infrared exposure employed with 20 control animals receiving ordinary white light for the same period that the infrared was given to treated animals.

The X-radiation was obtained from a General Electric Model OX-250 industrial unit with an S.R.T.-2 medical type tube, operating at 15 MA, 250 KV and having an inherent filtration of three mm of aluminum. Two additional millimeters of aluminum were added. All radiations were adminstered at 75r per minute (measured in air). Temperature at the time of X-radiation did not exceed 20.5° C. Point or spot X-radiation was employed on the tumor. The remaining parts of the animal were protected by lead ⅛ of an inch thick. Animal tumors receiving X-radiation alone were given varying initial high doses plus varying daily fractionated doses every 24 hours thereafter, for total dosages of 525, 1050, 1575, 2100, 2625, and 3150r (See Table II for methods of administration). Twenty animals were used for each dosage employed with 20 animals employed as untreated controls.

In the combined infrared X-radiation studies 20 animals bearing tumors were treated for each dosage of infrared employed. A constant dosage of fractionated X-radiation of 1050r was used in combination with 36, 48, 60, 72, 84, 96, and 108 hours of infrared radiation.

TABLE I. Number of regressions of tumor transplants from varying exposures to fractionated infrared radiation

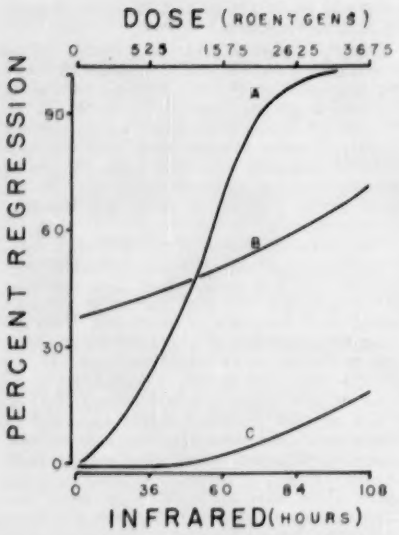
	Infrared Radiation			
No. of Animals	Pre-treatment (hours)	Fractional (hours)	Total (hours)	No. of Regressions
20*	0	0	0	0/20
20	24	3 hr × 4	36	0/20
20	24	6 hr × 4	48	0/20
20	24	9 hr × 4	60	1/20
20	24	12 hr × 4	72	2/20
20	24	15 hr × 4	84	2/20
20	24	18 hr × 4	96	1/20
20	24	21 hr × 4	108	4/20

*Control.

TABLE II. Number of regressions of tumor transplants from varying dosages of fractionated X-radiation

No of Animals	Initial Dose (r)	Fractionated Dose (r)	Total Dose (r)	No. of Regressions
20*	0	0	0	0/20
20	375	37.5r × 4	525	4/20
20	750	75.0r × 4	1050	7/20
20	1125	112.5r × 4	1575	13/20
20	1500	150.0r × 4	2100	17/20
20	1875	187.5r × 4	2625	20/20
20	2250	225.0r × 4	3150	20/20

*Control.



PERCENTAGE REGRESSIONS
Figure 21

A—Percentage tumor regressions with varying dosages of fractional X-radiation. *B*—Percentage tumor regressions with the X-radiation dose kept constant at 1050r and combined with varying exposures to infrared radiation. *C*—Percentage tumor regressions with fractionated infrared radiation given alone.

The infared was given as a pre-treatment to X-radiation at all times. Both the X-radiation doses of 1050r and the varying exposures to infrared radiations were administered as for the group receiving infrared or X-radiation alone (Table III).

Previous to undergoing X-radiation the animals were anesthetized with Nembutal sodium (Abbott) injected intraperitoneally at a dose equivalent to three mg per gm body weight. Control animals received the same anesthetic and were placed under the X-ray beam but completely shielded by lead while the machine was run for the same period of time as for the treated animals.

All animals were sacrificed at the end of a six week period post-irradiation. The number of animals showing absence of tumors was then recorded. The number of regressions were converted to percentages and the results graphed. The curves in Figure 21 were drawn to suit the points, but no attempt was made to fit the curve by statistical methods.

Results and Discussion

Infrared Controls. None of the control animals survived the six week period of the experiment. At the end of the two weeks 10 of the 20 were dead with tumors measuring on the average 2.8 cm in length by 1.5 cm in diameter. By five weeks the remaining 10 animals had died bearing tumors averaging 3.5 cm in length and 1.6 cm in diameter (Table I).

Infrared radiation therapy. In all cases of infrared exposures, the irradiated transplants grew as fast as the controls and in many cases, particularly at low exposures, faster during the first two weeks. The possible stimulatory effect on the tumor was particularly marked for exposure times of 36, 48, 60, and 72 hours. At four weeks the irradiated transplants became separable into two categories: those in which growth continued and those in which growth slowed down and regressions occurred. The former died four to five weeks after the commencement of the experiment. In the latter category, regressions occurred from five to six weeks after the start of the experiment, as revealed by gross examination. With increasing exposure times to infrared radiation from 60 to 108 hours an increased number of tumor regressions was recorded (Table I, Figure 21).

X-radiation therapy. Fractionated dosages of 525r, 1050r, 1575r, 2100r, 2625r, and 3150r of X-radiation were employed on the tumor. Complete regression of the tumor was evidenced in all animals receiving 3150r and 2625r. In a descending scale of effectiveness 2100r, 1575r, 1050r, and 525r caused a smaller percentage of regressions (Table II, Figure 21). No regressions were seen in the controls and at five weeks none of them had survived.

At a dosage of 525r, a possible stimulatory effect on the tumor was noted as evidenced by a rapid growth of the tumor in the first four weeks of the experiment. In some cases the tumors exceeded the control tumors in size, particularly in the first two weeks after the start of the experiment.

Combined infrared X-radiation therapy. In a previous paper it has been reported that 1050r of fractionated X-rays alone produces 33 percent tumor regressions.[1] In this experiment 35 percent of the tumors regressed with an exposure of 1050r of X-rays. With a com-

TABLE III. Number of regressions of tumor transplants from combined exposures to fractionated infrared radiation and fractionated X-radiation

No. of Animals	Infrared (hours)	Total (hours)	X-radiation (r)	Total (r)	No. of Regressions
20	24+ 1hr × 4	36	750+75r × 4	1050	7/15*
20	24+ 6hr × 4	48	750+75r × 4	1050	9/17
20	24+ 9hr × 4	60	750+75r × 4	1050	7/14
20	24+12hr × 4	72	750+75r × 4	1050	10/19
20	24+15hr × 4	84	750+75r × 4	1050	12/19
20	24+18hr × 4	96	750+75r × 4	1050	12/18
20	24+21hr × 4	108	750+75r × 4	1050	15/19

*Some of the original 20 animals in each group died from anesthesia. All groups received 1050r of fractionated X-rays given as an initial dose of 750r followed by four doses of 75r at 24-hour intervals. All groups received initially 24 hours of infrared radiation followed by daily dosages as indicated. Infrared was given as a pre-treatment at all times.

bination of 36 hours of fractionated infrared and 1050r of X-radiation seven out of 15 treated tumors had regressed upon completion of the experiment at six weeks (Table III, (Figure 21). With increasing duration of exposures up to 108 hours of fractionated infrared radiation, there was an increasing number of tumor regressions.

Food and water uptake were higher in the treated group than in the control group during the period of infrared radiation exposure. This has also been observed in non-tumor bearing animals exposed to infrared radiation for prolonged periods of time.[5]

With infrared exposures of 72, 84, 96, and 108 hours combined with 1050r of X-radiation there is a rise in the curve for the percentage of tumor regressions. With those groups receiving 72 hours' exposure to infrared, a time of 12 hours separated the exposures to infrared radiation on successive days. Shorter intervals were involved at 84, 96, and 108 hours respectively. The X-radiation was given within one hour after each exposure to infrared. Previous data have shown that infrared post-treatment may be given as much as 96 hours after X-radiation and still result in increases in chromosome aberrations.[21] This effective period may be shorter in tumor tissue and of a relatively few hours' duration. A plausible explanation for the rise in the percentage of tumor regressions with 72 hours or more of exposure to infrared may be that we are essentially pre- and post-treating the tumor tissue with infrared radiation resulting in a greater amount of damage to the tumor cells. Kaufmann, Hollaender and Gay[11] found that the effects of a combination of infrared and 4000r of X-radiation pre- or post-treatment on *Drosophila* sperm modified the frequency of chromosomal aberrations. A higher frequency of rearrangements was found with pre-treatment. It is not unlikely that a somewhat similar effect is being produced in the chromosomes of the tumor cells undergoing combined infrared and X-radiation therapy. Chromosomal rearrangements could conceivably produce an imbalance in the tumor cells resulting in their death. It should also be noted that tumors receiving either long exposures to infrared radiation or combined infrared and X-radiation exhibit a high degree of necrosis in the central portion of the tumor. This was not in evidence in any of the control tumors examined. The untreated tumor seldom exhibits any degree of necrosis.

Another possible explanation for an increase in tumor regressions where longer exposures to infrared are involved may tie in with the recovery period in the tumor tissue. At lower dosages in the infrared the time interval between successive exposures to infrared is longer, allowing for possible recovery of the tumor cells. At higher dosages in the infrared, where shorter intervals between exposures to the infrared are involved the tissue may not have recovered when the next exposure commences. This could conceivably do more damage to the tissues concerned.

External heat is kept at a minimum because of the filters used with the infrared equipment. We can not, however, exclude the fact that there may be a translation of radiant energy to heat energy within the tissues. In the infrared region of the spectrum, vibrational or rotational energy levels are activated. Excited molecules may revert to normal or ground state through the release or re-emission of energy; this release may be in the form of heat, mechanical energy, or in some instances as radiant energy appearing as fluorescence or phosphorescence.

The simplest molecules, such as H_2O, CO_2, etc., require large energy values to be excited electronically and hence their absorbing region will be in the extreme ultraviolet or shortwave region. With increasing complexity of the molecules the amount of energy required to excite the molecule electronically is decreased; therefore, by progressive increases in molecular complexity, the wave length of the absorbed radiation characteristic of the molecule is shifted to longer wave length values, through the ultraviolet and across the visible spectrum to the red end[4]. The possibility that certain molecules or molecular systems within the cell are affected by the relatively low energies involved with infrared radiation cannot be excluded.

Another possible mechanism of effect of infrared and of combined infrared and X-radiation on tumors may be on the blood supply to the tumor. Earle[7,8] reported a rapid degeneration of leucocytes from the blood of cats, rabbits, etc., in tissue culture after exposure to light in the blue, green, and infrared regions. A somewhat similar phenomenon had been observed in our laboratory when non-tumor bearing mice are exposed to continuous infrared radiation for periods up to 576 hours.[5] The initial results would indicate lower hematocrits and hemoglobins as well as an effect on the femoral bone marrow in mice subjected to prolonged exposures of infrared radiation, as evidenced by a depletion of the marrow in the treated animals.

From the data presented the effects of infrared radiation alone and combined with X-radiation still lie in the realm of speculation. It is felt, however, that the evidence would tend to indicate both a physiological and physical effect on the cell and its constituents.

Summary and Conclusions

Total body exposures to infrared radiation of 36, 48, 60, and to some extent 72 hours have a probable stimulatory effect on the mouse tumor studied. With increasing exposures of 72, 84, 96, and 108 hours there is an increased number of tumor regressions.

An X-radiation dose of 525r has a possible stimulatory effect on the tumor studied. Complete regression of the tumor takes place at

dosages of 3150r and 2625r. With decreasing dosages there is a decreased number of tumor regressions attained.

Fractionated infrared radiation in the spectral region of 10,000 A° is an effective potentiating agent when used in combination with fractionated X-radiation when the criterion of effect is the number of tumor regressions produced by the combined therapy on the F 191 mammary adenocarcinoma in mice.

When the fractionated X-radiation dose of 1050r is kept constant and the exposure to fractionated infrared radiation is varied from 36 to 108 hours there is an increasing number of tumor regressions proportional to the increased exposure to infrared radiation. When 96 hours of fractionated infrared radiation is combined with 1050r of fractionated X-radiation the effectiveness of the X-radiation in producing tumor regressions is doubled.

At infrared exposures of 72 to 108 hours there is an apparent rise in the curve for tumor regressions which may be explainable on the basis of a combined pre- and post-treatment to X-radiation hypothesis and/or a recovery factor may be involved in the tumor tissue.

Possible mechanisms of the effect of infrared radiation on the cell are discussed.

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